

Modulating Evolutionary Potential with Rationally Designed Genes

Jarrod Shilts

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The possibility of mutation represents a fundamental limit to the widespread application of genetically engineered system. The aims of this project are to 1) quantify the selective pressures acting on mutant alleles in synthetic biology applications 2) invent a method for designing genes with altered susceptibility to mutation 3) demonstrate that sequence engineering is an effective means of changing evolutionary potential and 4) introduce additional genetic modifications to modify the frequency of mutation

Project track:

- **Past Milestones**

- UV RFP Growth Curves
 - Experiment: 2015-05-17 Setting up cell plates
- RFP+K395 Competition
 - Experiment: 2015-05-28 Setting up experiment
 - Experiment: 2015-05-29 Measurement and re-plating
 - Experiment: 2015-06-05- Final protocol changes
- Second RFP Competition Test
 - Experiment: 2015-06-11 Setting up experiment
 - Experiment: 2015-06-11 Cell Counting
- Cloning YY Optimized RFP
 - Experiment: 2015-05-31 Inserting promoter
 - Experiment: 2015-05-23 RFP gBlock into pSB1C3
 - Experiment: 2015-05-31 PCR Confirm Ligation
 - Experiment: 2015-06-01 Digest Confirmation of ligation
 - Experiment: 2015-06-05 PCR Confirm Ligation
 - Experiment: 2015-06-06 Second transformation of 5/23 ligations
 - Experiment: 2015-06-06 gBlock digest and ligation
 - Experiment: 2015-06-10 Ligation Confirmation

- Experiment: 2015-06-13 Adding promoter
- Revised GFP UV Test
 - Experiment: 2015-06-01 Setting up plates (old protocol)
 - Experiment: 2015-06-01 - Setting up plates (new protocol)
- Optimizing T4 PDG Assay
 - Experiment: 2015-06-20 PCR Extraction
 - Experiment: 2015-06-22 T4 PDG Reaction
 - Experiment: 2015-06-23 T4 PDG Reaction
 - Experiment: 2015-06-26 PCR Extraction 2
 - Experiment: 2015-06-26 T4 PDG Reaction
 - Experiment: 2015-06-28 PDG Nicked Plasmid
 - Experiment: 2015-07-01 Overnight PDG incubation
- Bulky DNA Lesion PCR
 - Experiment: 2015-07-06 UV irradiated PCR
 - Experiment: 2015-07-07 PDG PCR
 - Experiment: 2015-07-08 UV PCR
- RFPyy Expression Tests
 - Experiment: 2015-06-20 Heterogeneity in RFP expression
 - Experiment: 2015-06-27 Making Competent BL21 Cells
 - Experiment: 2015-06-28 Testing Competence and RFP expression
 - Experiment: 2015-06-28 Making Competent DH5 alpha Cells
 - Experiment: 2015-06-29 DH5 alpha transformations
 - Experiment: 2015-07-01 - Promoter Miniprep
 - Experiment: 2015-07-02 Adding R0010 promoter to RFPyy
- Spectrophotometer OD/RFP Calibration
 - Experiment: 2015-06-12 Setting up dilutions
 - Experiment: 2015-06-12 Counting colonies
- T4 PDG Assay for RFPyy
 - Experiment: 2015-07-02 T4 PDG with UV of 100 and 1000 mJ/cm²
 - Experiment: 2015-07-04 T4 PDG at 50 and 500 mJ/cm²
 - Experiment: 2015-07-06 PDG Assay, 4 replicates

- **Current Milestones**

- Optimized Part Collection
 - Experiment: 2015-09-14 gBlock ligation
- T7 Ura3 Homology Assembly
 - Experiment: 2015-08-24 T7 and Ura3 PCR
 - Experiment: 2015-08-25 Extractions

- VERT Assembly
 - Experiment: 2015-09-04 Vector extraction
 - Experiment: 2015-09-07 Gibson assembly confirmation
- Part Expression
 - Experiment: 2015-08-22 RFP T7 ligation
 - Experiment: 2015-08-26 T7 RFP expression
 - Experiment: 2015-08-28 T7 Ligation Check
 - Experiment: 2015-09-06 J04500 promoter assembly
 - Experiment: 2015-09-15 S1 T7 Coomassie
- RFP uv Experimentation
 - Experiment: 2015-08-03 RFP uv PCR
 - Experiment: 2015-08-18 RFP uv Extraction
 - Experiment: 2015-08-21 RFP uv alkaline gel
 - Experiment: 2015-09-12 RFPuv + RFPOx alkaline gel
- gBlock to Biobrick in pSB
 - Experiment: 2015-07-21 gBlock PCR
 - Experiment: 2015-07-22 gBlock Ligations
 - Experiment: 2015-07-25 gBlock+ pSB1C3 confirmation
 - Experiment: 2015-07-26 Promoter ligations and redo gBlock extractions
 - Experiment: 2015-07-27 ligation transformations
 - Experiment: 2015-07-30 ligation confirmation
 - Experiment: 2015-08-18 RFP ox, S1, pKIKO confirmation
 - Experiment: 2015-08-19 Biobrick sequencing
 - Experiment: 2015-08-22 Re-acquiring assemblies
 - Experiment: 2015-08-29 Ligation confirmation
- Ura3 Recombination
 - Experiment: 2015-08-07 - Genomic DNA Extraction
 - Experiment: 2015-08-09 - PCR Amplification of URA3 Insert
 - Experiment: 2015-08-10 - pUC19/URA3 Insert Digestion with BamHI-HF, SpeI
 - Experiment: 2015-08-11 - Gel Extraction of pUC19/URA3 Digest
 - Experiment: 2015-08-27 Ligation Check
 - Experiment: 2015-09-03 Second round ura3 PCR
 - Experiment: 2015-09-01 first round ura PCR
- FPG Oxidation Assay
 - Experiment: 2015-07-09 FPG methylene blue Test
 - Experiment: 2015-07-14 FPG methylene blue
 - Experiment: 2015-07-16 FPG peroxide oxidation

- Experiment: 2015-07-21 Plasmid Oxidation FPG Nick
- Experiment: 2015-08-20 RFP ox oxidation assay
- Experiment: 2015-08-22 fpg oxidation
- Experiment: 2015-09-04 RFPox oxidation
- Experiment: 2015-09-05 Oxidation new RFP ox
- **Future Milestones**

Project Attached Files

DNA_Damage_-_Detection_Strategies.pdf

Notes

- **Constructed Parts:**

Catalog Number	Component Parts	Description
BBa_K1673000	RFP(yy)	RFP optimized for pyrimidine dimers
BBa_K1673001	K314100+RFP(yy)	RFP(yy) with a constitutive promoter
BBa_K1673002	K314100+E1010	RFP with a constitutive promoter
BBa_K1673003		



Milestone: **UV RFP Growth Curves**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Mutagenize J04450 RFP (with constitutive promoter) at UV dosages <500 J/M² to determine if higher UV dosage correlates with a greater change in fluorescence

Experiment: **2015-05-17 Setting up cell plates**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: UV RFP Growth Curves | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
51				

Steps

#	Title	Timer																																																																														
1	Transform and grow 5 liquid cultures of J04450 RFP with constitutive promoter for ~24 hours	00:00:00																																																																														
2	Add 250 ul of cells to well of 96 well plate according to table below. Each row is a different starting liquid culture, and each column is a different UV dosage. First two wells have no UV. Once aliquoted, expose liquid culture tubes to 1 J/m2 of UV. Aliquot, then expose to 4 J/m2 more UV. Aliquot, then expose to 5 J/m2 more UV. Aliquot, then expose 10 J/m2 more UV. Aliquot, then expose 10 J/m2 more UV. Aliquot, then expose 20 J/m2 more UV. Aliquot, then expose 50 J/m2 more UV. Aliquot, then expose 100 J/m2 more UV. Aliquot, then expose 300 J/m2 more UV	00:00:00																																																																														
	<table border="1"> <thead> <tr> <th></th> <th>1 (0J/ m2)</th> <th>2 (0J/ m2)</th> <th>3(1 J/ m2)</th> <th>4 (5 J/ m2)</th> <th>5 (10 J/ m2)</th> <th>6 (20 J/ m2)</th> <th>7 (30 J/ m2)</th> <th>8 (50 J/ m2)</th> <th>9 (100 J/m2)</th> <th>10 (200 J/m2)</th> <th>11 (500 J/ m2)</th> <th>12 (Blank)</th> </tr> </thead> <tbody> <tr> <td>A (liquid culture A)</td> <td>0</td> <td>0</td> <td>1</td> <td>5</td> <td>10</td> <td>20</td> <td>30</td> <td>50</td> <td>100</td> <td>200</td> <td>Empty</td> <td>Empty</td> </tr> <tr> <td>B (liquid culture B)</td> <td>0</td> <td>0</td> <td>1</td> <td>5</td> <td>10</td> <td>20</td> <td>30</td> <td>50</td> <td>100</td> <td>200</td> <td>500</td> <td>Empty</td> </tr> <tr> <td>C (liquid culture C)</td> <td>0</td> <td>0</td> <td>1</td> <td>5</td> <td>10</td> <td>20</td> <td>30</td> <td>50</td> <td>100</td> <td>Empty</td> <td>Empty</td> <td>Empty</td> </tr> <tr> <td>D (liquid culture D)</td> <td>0</td> <td>0</td> <td>1</td> <td>5</td> <td>10</td> <td>20</td> <td>30</td> <td>50</td> <td>100</td> <td>200</td> <td>LB+CMR Blank</td> <td>LB+CMR Blank</td> </tr> <tr> <td>E (liquid culture E)</td> <td>0</td> <td>0</td> <td>1</td> <td>5</td> <td>10</td> <td>20</td> <td>30</td> <td>50</td> <td>100</td> <td>200</td> <td>LB+CMR Blank</td> <td>LB+CMR Blank</td> </tr> </tbody> </table>		1 (0J/ m2)	2 (0J/ m2)	3(1 J/ m2)	4 (5 J/ m2)	5 (10 J/ m2)	6 (20 J/ m2)	7 (30 J/ m2)	8 (50 J/ m2)	9 (100 J/m2)	10 (200 J/m2)	11 (500 J/ m2)	12 (Blank)	A (liquid culture A)	0	0	1	5	10	20	30	50	100	200	Empty	Empty	B (liquid culture B)	0	0	1	5	10	20	30	50	100	200	500	Empty	C (liquid culture C)	0	0	1	5	10	20	30	50	100	Empty	Empty	Empty	D (liquid culture D)	0	0	1	5	10	20	30	50	100	200	LB+CMR Blank	LB+CMR Blank	E (liquid culture E)	0	0	1	5	10	20	30	50	100	200	LB+CMR Blank	LB+CMR Blank	
	1 (0J/ m2)	2 (0J/ m2)	3(1 J/ m2)	4 (5 J/ m2)	5 (10 J/ m2)	6 (20 J/ m2)	7 (30 J/ m2)	8 (50 J/ m2)	9 (100 J/m2)	10 (200 J/m2)	11 (500 J/ m2)	12 (Blank)																																																																				
A (liquid culture A)	0	0	1	5	10	20	30	50	100	200	Empty	Empty																																																																				
B (liquid culture B)	0	0	1	5	10	20	30	50	100	200	500	Empty																																																																				
C (liquid culture C)	0	0	1	5	10	20	30	50	100	Empty	Empty	Empty																																																																				
D (liquid culture D)	0	0	1	5	10	20	30	50	100	200	LB+CMR Blank	LB+CMR Blank																																																																				
E (liquid culture E)	0	0	1	5	10	20	30	50	100	200	LB+CMR Blank	LB+CMR Blank																																																																				

3	With fluorometer, measure Absorbance 600 and Fluorescence 530/25, 645/40 (~8pm)	00:00:00
4	Wrap in parafilm and place on 37 degree shaking incubator. (Note: this step was skipped for the first 24 hours, which may have caused the visible loss of volume in some wells)	00:00:00

Milestone: **RFP+K395 Competition**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Owner: **Jarrod Shilts**

Experiment: **2015-05-28 Setting up experiment**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **RFP+K395 Competition** | Owner: **Jarrod Shilts**

Experiment Procedures

First procedure

Steps

#	Title
1	Transform cells with K395602 (apple scent generator without a promoter) and J04450 (RFP with constitutive strong Plate on CMR+LB, and make 5ml liquid cultures after ~24 hrs

2

Set up competition experiment

Set up 3 ml liquid cultures x5

100% RFP, 0% K395	90% RFP, 10% K395	75% RFP, 25% K395	50% RFP, 50% K395	25% RFP, 75% K395
-1 ml fresh LB+CMR -2 ml J04450	-1 ml fresh LB+CMR -1.8 ml J04450 -0.2 ml K395602	-1 ml fresh LB+CMR -1.5 ml J04450 -0.5 ml K395602	-1 ml fresh LB+CMR -1 ml J04450 -1 ml K395602	-1 ml fresh LB+CMR -0.5 ml J04450 -1.5 ml K395602

Set up 96 well plate, two rows of 10 at 250 ml each well

	1	2	3	4	5	6	7	8	9	10	11
A	99%	95%	90%	80%	70%	60%	50%	40%	30%		
	100% RFP	RFP	RFP	RFP	RFP	RFP	RFP	RFP	RFP		
	RFP -100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	
	-100 ul fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	
	LB+CMR -148 ul	LB+CMR	Empt								
	-148 ul J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	
	-150 ul J04450	-2 ul	-8 ul	-15 ul	-30 ul	-45 ul	-60 ul	-75 ul	-60 ul	-45 ul	
	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	
B	99%	95%	90%	80%	70%	60%	50%	40%	30%		
	100% RFP	RFP	RFP	RFP	RFP	RFP	RFP	RFP	RFP		
	RFP -150 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	-100 ul	
	-150 ul fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	
	LB+CMR -99 ul	LB+CMR	Empt								
	-99 ul J04450	-95 ul	-90 ul	-80 ul	-70 ul	-60 ul	-50 ul	-40 ul	-30 ul		
	-100 ul J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	J04450	
	J04450 -1 ul	-5 ul	-10 ul	-20 ul	-30 ul	-40 ul	-50 ul	-60 ul	-70 ul		
	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	K395602	

3	Add 200 ul of the 3ml cultures to 96 well plate for measurement								
	1	2	3	4	5	6	7	8	9
C	100% RFP liquid culture	90% RFP liquid culture	75% RFP liquid culture	50% RFP liquid culture	25% RFP liquid culture				
4	On fluorometer, measure Absorbance 600nm and Fluorescence 530/25, 645/40 (roughly 9pm)								
5	Wrap plate in parafilm. Place plate and liquid cultures on 37 degree shaking incubator for ~24 hours								

Samples & Reagents

Id	Type	Name	Tube	Remarks
21				

Experiment: **2015-05-29 Measurement and re-plating**

**Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFP+K395
Competition | Owner: Jarrod Shilts**

Procedure for measuring fluorescence changes in experimental populations, and preparing cultures for the following day

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
41				

Steps

#	Title																										
1	<p>Add 200 ul of the liquid cultures to the 96 well plate. Mix gently if any sedimentation of cells</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td></td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td>5</td> <td>6</td> <td>7</td> <td>8</td> <td>9</td> <td>10</td> <td>11</td> <td>12</td> </tr> <tr> <td>C</td> <td>Old 100% RFP culture</td> <td>Old 90% RFP culture</td> <td>Old 75% RFP culture</td> <td>Old 50% RFP culture</td> <td>Old 25% RFP culture</td> <td>Empty</td> <td>Empty</td> <td>200 ul new 100% RFP culture</td> <td>200 ul new 90% RFP culture</td> <td>200 ul new 75% RFP culture</td> <td>200 ul new 50% RFP culture</td> <td>200 ul new 25% RFP culture</td> </tr> </table>		1	2	3	4	5	6	7	8	9	10	11	12	C	Old 100% RFP culture	Old 90% RFP culture	Old 75% RFP culture	Old 50% RFP culture	Old 25% RFP culture	Empty	Empty	200 ul new 100% RFP culture	200 ul new 90% RFP culture	200 ul new 75% RFP culture	200 ul new 50% RFP culture	200 ul new 25% RFP culture
	1	2	3	4	5	6	7	8	9	10	11	12															
C	Old 100% RFP culture	Old 90% RFP culture	Old 75% RFP culture	Old 50% RFP culture	Old 25% RFP culture	Empty	Empty	200 ul new 100% RFP culture	200 ul new 90% RFP culture	200 ul new 75% RFP culture	200 ul new 50% RFP culture	200 ul new 25% RFP culture															
2	Measure Absorbance 600nm and Fluorescence 530/25, 645/40 on fluorometer (~8pm)																										
3	Renew 3ml liquid cultures by adding 1 ml fresh LB+CMR to tube and then adding 2 ml of cells from prior day's liquid																										

4

Re-plate by adding to rows of 96 well plate below previous ones

	1	2	3	4	5	6	7	8	9	10	11	12
D	100% RFP -100 ul of A1	99% RFP -100 ul of A2	95% RFP -100 ul of A3	90% RFP -100 ul of A4	80% RFP -100 ul of A5	70% RFP -100 ul of A6	60% RFP -100 ul of A7	50% RFP -100 ul of A8	40% RFP -100 ul of A9	30% RFP -100 ul of A10	Empty	
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-150 ul of A1	-150 ul of A2	-150 ul of A3	-150 ul of A4	-150 ul of A5	-150 ul of A6	-150 ul of A7	-150 ul of A8	-150 ul of A9	-150 ul of A10		
	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh		
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-150 ul of B1	-150 ul of B2	-150 ul of B3	-150 ul of B4	-150 ul of B5	-150 ul of B6	-150 ul of B7	-150 ul of B8	-150 ul of B9	-150 ul of B10		
	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh		
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-100 ul of B1	-100 ul of B2	-100 ul of B3	-100 ul of B4	-100 ul of B5	-100 ul of B6	-100 ul of B7	-100 ul of B8	-100 ul of B9	-100 ul of B10		
	of A1	of A2	of A3	of A4	of A5	of A6	of A7	of A8	of A9	of A10		
E	100% RFP -150 ul of B1	99% RFP -150 ul of B2	95% RFP -150 ul of B3	90% RFP -150 ul of B4	80% RFP -150 ul of B5	70% RFP -100 ul of B6	60% RFP -100 ul of B7	50% RFP -100 ul of B8	40% RFP -100 ul of B9	30% RFP -100 ul of B10	Empty	
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-100 ul of B1	-100 ul of B2	-100 ul of B3	-100 ul of B4	-100 ul of B5	-100 ul of B6	-100 ul of B7	-100 ul of B8	-100 ul of B9	-100 ul of B10		
	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh		
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-100 ul of A1	-100 ul of A2	-100 ul of A3	-100 ul of A4	-100 ul of A5	-100 ul of A6	-100 ul of A7	-100 ul of A8	-100 ul of A9	-100 ul of A10		
	of A1	of A2	of A3	of A4	of A5	of A6	of A7	of A8	of A9	of A10		
	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR	LB+CMR		
	-150 ul of A1	-150 ul of A2	-150 ul of A3	-150 ul of A4	-150 ul of A5	-150 ul of A6	-150 ul of A7	-150 ul of A8	-150 ul of A9	-150 ul of A10		
	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh	fresh		

5

Replenish row A by adding 150 ul of fresh LB+CMR to all wells where cells were withdrawn (A1-A10)

Replenish row B by adding 100 ul of fresh LB+CMR to all wells where cells were withdrawn (B1-B10)

6

Add 200 ul of the 3ml cultures to 96 well plate for measurement

	1	2	3	4	5	6	7	8	9	10
D	(New) 100% RFP liquid culture	(New) 90% RFP liquid culture	(New) 75% RFP liquid culture	(New) 50% RFP liquid culture	(New) 25% RFP liquid culture					

7

Measure Absorbance 600nm and Fluorescence 530/25, 645/40 on fluorometer (~9pm)

8

Wrap plate in parafilm. Place plate and liquid cultures on 37 degree shaking incubator for ~24 hours

Experiment: **2015-06-05- Final protocol changes**

**Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFP+K395
Competition | Owner: Jarrod Shilts**

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
131				

Steps

#	Title	Timer
1	After observing a greater than expected decrease in fluorescence over time in the control strain (100% J04450, 0% K395), it was hypothesized that the culture volumes used (1 ml new culture+antibiotic, 2 ml old culture) were generating insufficient selective pressure to force the cells to maintain their plasmid. Thus, after measurements were taken, 3 ul of cells were added to 3 ml LB + 4 ul CMR	00:00:00
2	After 24 hours growth, measurements were taken. A 1:10^6 dilution was done on 100 J 0 K and 90 J 10 K and 100 ul of the dilution was plated on CMR plates	00:00:00
3	Measure fluorescence. Because cells (including control) not yet visibly red, growth continued for another 24 hours. 200 ul of 100%J, 0%K and 200 ul of 90%J, 10%K plated on CMR plates	00:00:00
4	Re-streak 100J 0K plate to isolate individual red colony to restart experiment. No red found on 90J 10K lawn	00:00:00

Milestone: **Second RFP Competition Test**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Type here the description for this folder, what experiments are to be done and what figures are expected

Experiment: **2015-06-11 Setting up experiment**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Second RFP Competition Test | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
201				

Steps

#	Title	Timer																		
1	Grow 3 ml liquid of clonal J04450 RFP and K395 generator without promoter. After 24 hours growth, put 200 ul on 96 well plate as zero point	00:00:00																		
2	Make six 3ml liquid cultures+chloramphenicol, vortexing before each dilution: <table border="1" style="margin-left: auto; margin-right: auto;"> <tr> <td>0 K395</td><td>10^6 K395</td><td>10^4 K395</td><td>10^2 K395</td><td>10^0 K395</td><td>K395</td></tr> <tr> <td>200 ul</td><td>197 ul J04450</td><td>190 ul J04450 + 10 ul of 10^6 K395</td><td>170 ul of J04450 + 30 ul of 10^4 K395</td><td>170 ul J04450 + 30 ul 10^2 K395</td><td>200 ul K395</td></tr> <tr> <td>J04450</td><td>+ 3 ul K395</td><td></td><td></td><td></td><td></td></tr> </table>	0 K395	10^6 K395	10^4 K395	10^2 K395	10^0 K395	K395	200 ul	197 ul J04450	190 ul J04450 + 10 ul of 10^6 K395	170 ul of J04450 + 30 ul of 10^4 K395	170 ul J04450 + 30 ul 10^2 K395	200 ul K395	J04450	+ 3 ul K395					00:00:00
0 K395	10^6 K395	10^4 K395	10^2 K395	10^0 K395	K395															
200 ul	197 ul J04450	190 ul J04450 + 10 ul of 10^6 K395	170 ul of J04450 + 30 ul of 10^4 K395	170 ul J04450 + 30 ul 10^2 K395	200 ul K395															
J04450	+ 3 ul K395																			
3	Incubate shaking at 250 rpm, 37 degrees for 24 hours between each subsequent measurement	00:00:00																		

Experiment: **2015-06-11 Cell Counting**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Second RFP Competition Test | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
211				

Steps

#	Title	Timer
1	Take 3ml liquid cultures from GFP 0 (at OD 0.94), K395 (at OD 1.11), and J04450 (at OD 1.40). Add 2 ul each culture to 1 ml LB. Then add 2 ul of dilution to 1 ml more LB. Plate 200ul as 1:10^6 dilution. Add 100 ul of dilution to 900 ul LB. Plate 200 ul as 1:10^7 dilution. (Note: plates used had sub-effective dose of chloramphenicol, which may interfere with GFP data point)	00:00:00
2	Let LB soak for 15 minutes, then place inverted in incubator	00:00:00
3		00:00:00

Count colonies that form after 24 hours of growth. For 1:10^6 dilutions, divide plate in half and double number.

Experiment Results

No data could be acquired for either GFP 0 plate, or J04450 1:10⁶.

J04450 1:10 ⁶	K395 1:10 ⁶	K395 1:10 ⁷
584	456	80

Although there are too few data points for accurate analysis, it can be estimated that a cell culture at OD = 1.0 has around 10⁸ to 10⁹ cells.

Milestone: Cloning YY Optimized RFP

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Create two vectors for UV comparison experiments, one with pyrimidine-dimer optimized RFP gBlock under K314100 constitutive promoter, and one with E1010 wild type RFP under

Experiment: 2015-05-31 Inserting promoter

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Steps

#	Title	Timer
1	Cut miniprepped K314100 with EcoRI and SpeI Cut miniprepped E1010 with EcoRI and XbaI Cut miniprepped RFPyy ligation 1A with EcoRI and XbaI Cut miniprepped RFPyy ligation 1B with EcoRI and XbaI For all reactions, 10 ul volume with cutsmart buffer and 1 ul of plasmid DNA (~100 ng)	00:00:00
2	Incubate 1 hour at 37 degrees. Purify vectors (E1010 and RFPyy ligations) with Promega Wizard DNA cleanup kit, and purify promoter (K314) by gel extraction.	00:00:00
3		00:00:00

Samples & Reagents

Id	Type	Name	Tube	Remarks
61				

Procedure Results:

Gel of digested promoter had band too faint for extraction

Experiment: **2015-05-23 RFP gBlock into pSB1C3**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
71				

Steps

#	Title	Timer
1	Digest 2 ul RFPyy gBlock in 5 ul water, 1 ul NEBuffer 2.1, 1.5 ul PstI, and 1 ul EcoRI-HF. Also digest 2 ul linearized pSB1C3 with same recipie	00:00:00
2	Incubate 37 degree for 2 hours, then heat inactivate 30 minutes at 80 degrees	00:00:00
3	Set up two ligation reactions with 5 ul gBlock, 3 ul linearized pSB1C3, 2 ul T4 Ligase, and 8.5 ul water. Incubate for 1 hour at room temperature	00:00:00
4	Thaw competent cells for 10 minutes on ice, then add 4 ul of ligation product. Also add 1 ul of K395602	00:00:00
5	Incubate cells on ice 30 minutes, heat shock 30 seconds at 42 degrees, ice recovery for 4 minutes, then add 300 ul of warmed LB. Outgrowth for 1 hour	00:00:00

Experiment: **2015-05-31 PCR Confirm Ligation**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

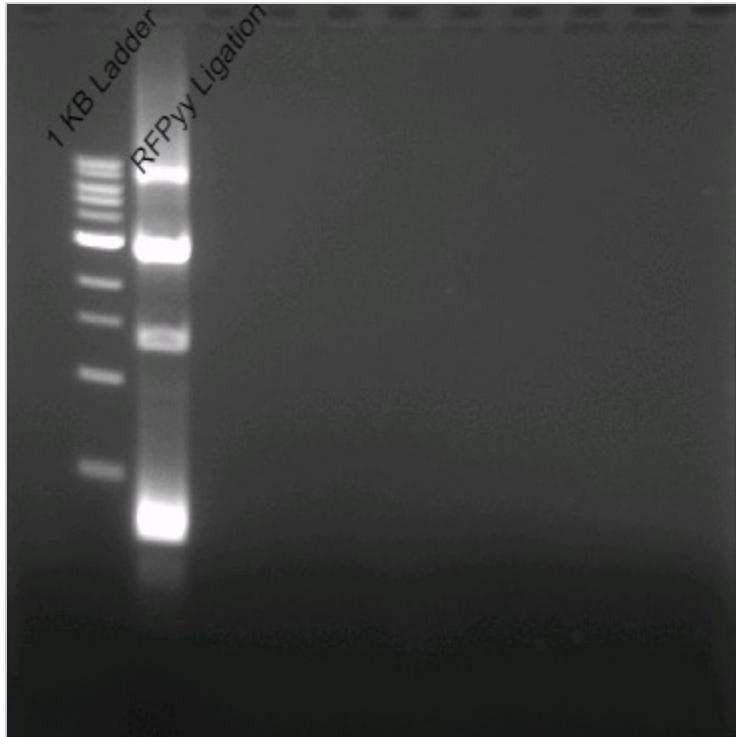
Id	Type	Name	Tube	Remarks
91				

Steps

#	Title	Timer
1	With Q5 Polymerase master mix, run PCR on RFPyy ligation 1B	00:00:00
2	Run gel and image	00:00:00
3		00:00:00

Experiment Results

Experiment Attached Images



05-31_RFP_yy_PCR_check.bmp

Experiment: 2015-06-01 Digest Confirmation of ligation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
101				

Steps

#	Title	Timer
1	Digest RFP yy lig 1B with Eco and Xba in one tube, and Eco and Pst in another. Also digest K395 generator part with Eco and Pst. Incubate 37 degrees for 1 hour	00:00:00
2	Run on 1% gel and image	00:00:00

Experiment: 2015-06-05 PCR Confirm Ligation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
121				

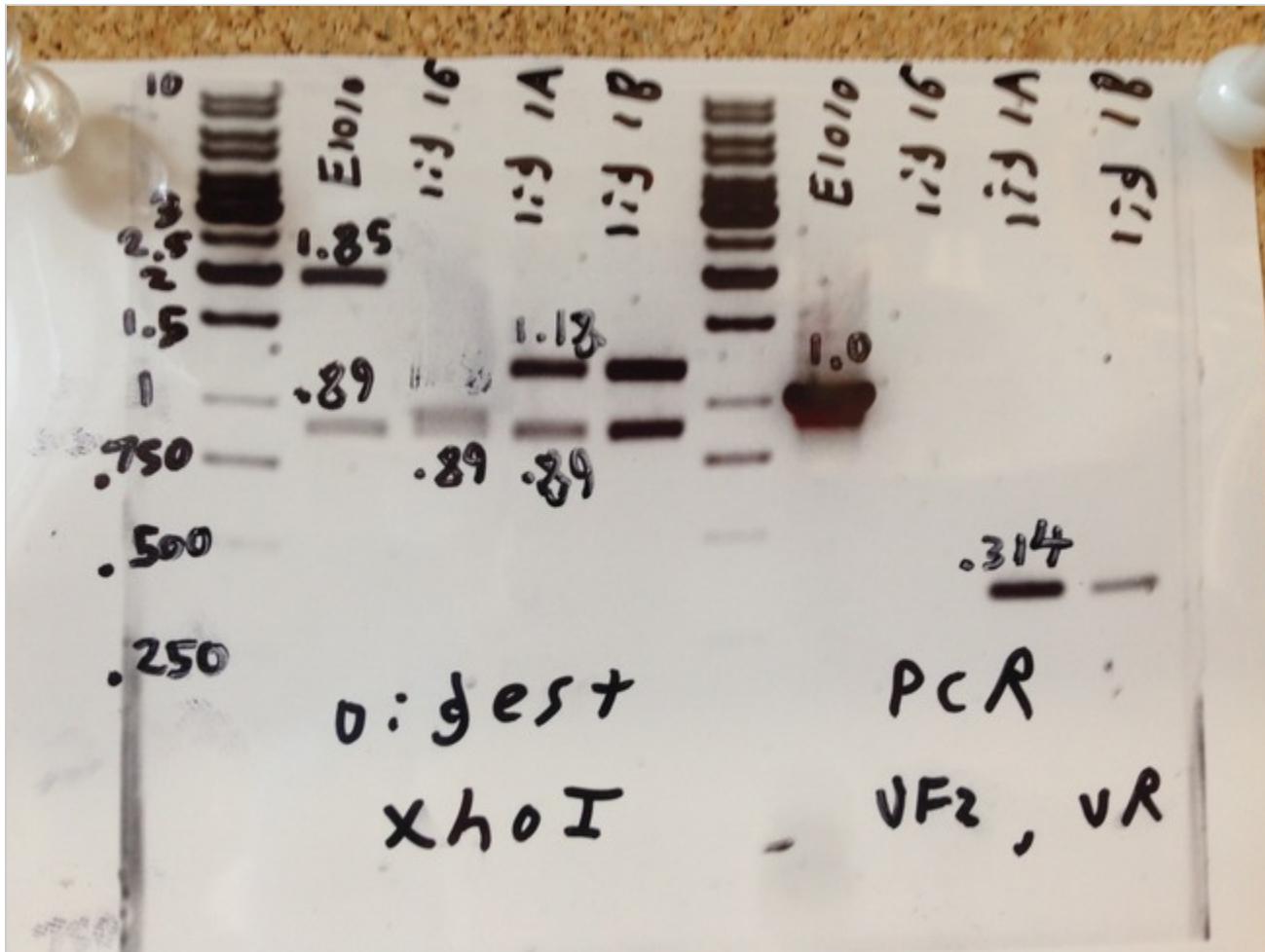
Steps

#	Title	Timer
1	With XhoI in cutsmart, digest lig 1B, lig 2A, and E1010	00:00:00
2	Using VF2 and VR primers, PCR insert region. 25 ul hot start Taq reaction (Ta=55 C) with 0.5 ul of plasmid added following dilution 1:50	00:00:00
3	Run 1.2 % gel on digest and PCR products	00:00:00

Experiment Conclusion

Positive control (E1010) gave expected bands. If ligation successful, ligation would appear very similar to E1010 results. Lig 5/16 appears to be a partial pSB1C3 with part near its insert region missing (removing primer site and reducing digest fragment size). Lig 1A and 1B are identical, and are self-ligated pSB1C3 with no insert

Experiment Attached Images



RFPyy_ligations_identification.JPG

Experiment: 2015-06-06 Second transformation of 5/23 ligations

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
141				

Steps

#	Title	Timer
1	Remove 5/23 ligation reactions from -20 degree freezer. Place competent cells on ice for 3 minutes. Add 5 ul of ligation reaction 1 to one aliquot of cells, and 5 ul ligation reaction 2 to second aliquot. To both, also add 1 ul DpnI	00:00:00
2	Mix by passing finger over bottom of tube. Keep on ice 30 minutes, heat shock 42 degree 1 minute, recover on ice 5 minutes, then add 250 ul of room temperature SOC media. Shake at 37 degrees for 1 hour	00:00:00
3	Add the entire reaction to pre-warmed 37 degree CMR plates. Spread with disposable spreaders	00:00:00

Experiment: **2015-06-06 gBlock digest and ligation**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Steps

#	Title	Timer
1	In a 25 ul reaction, digest 3 ul of RFPyy gBlock (at 10 ng/ul) with EcoRI-HF and PstI in NEBuffer 2.1. With same conditions, also digest 3 ul miniprepped J04450 (at 200 ng/ul) and 3ul linearized pSB1C3 (at 25 ng/ul). Incubate 37 degrees for 1.5 hours, then heat inactivate 80 degrees 30 minutes	00:00:00
2	Run digested J04450 on gel and gel extract linear band at 2.9 kb with Qiagen gel extraction kit. Yield around 6 ng/ul when eluted in 50 ul	00:00:00
3	In a 20 ul ligation reaction, add 13 ul digested gBlock (15.6 ng) to 4 ul digested linearized backbone (12 ng). In another reaction, add 12 ul digested gBlock (14.4 ng) to 3 ul gel-extracted backbone (18 ng)	00:00:00
4	Incubate 37 degrees for 15 minutes, then 16 degrees for 16 hours on thermocycler	00:00:00
5	Transform the two ligation reactions into two aliquots of competent cells. 30 min ice, 50 sec heat shock, 5 min ice, 60 min outgrowth	00:00:00

Experiment Results

>100 colonies seen on both plates. Both have a handful of red colonies from autoligation, residual PCR template, or contamination.

Experiment Conclusion

Total of 5 liquid cultures made from plates

Experiment: 2015-06-10 Ligation Confirmation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

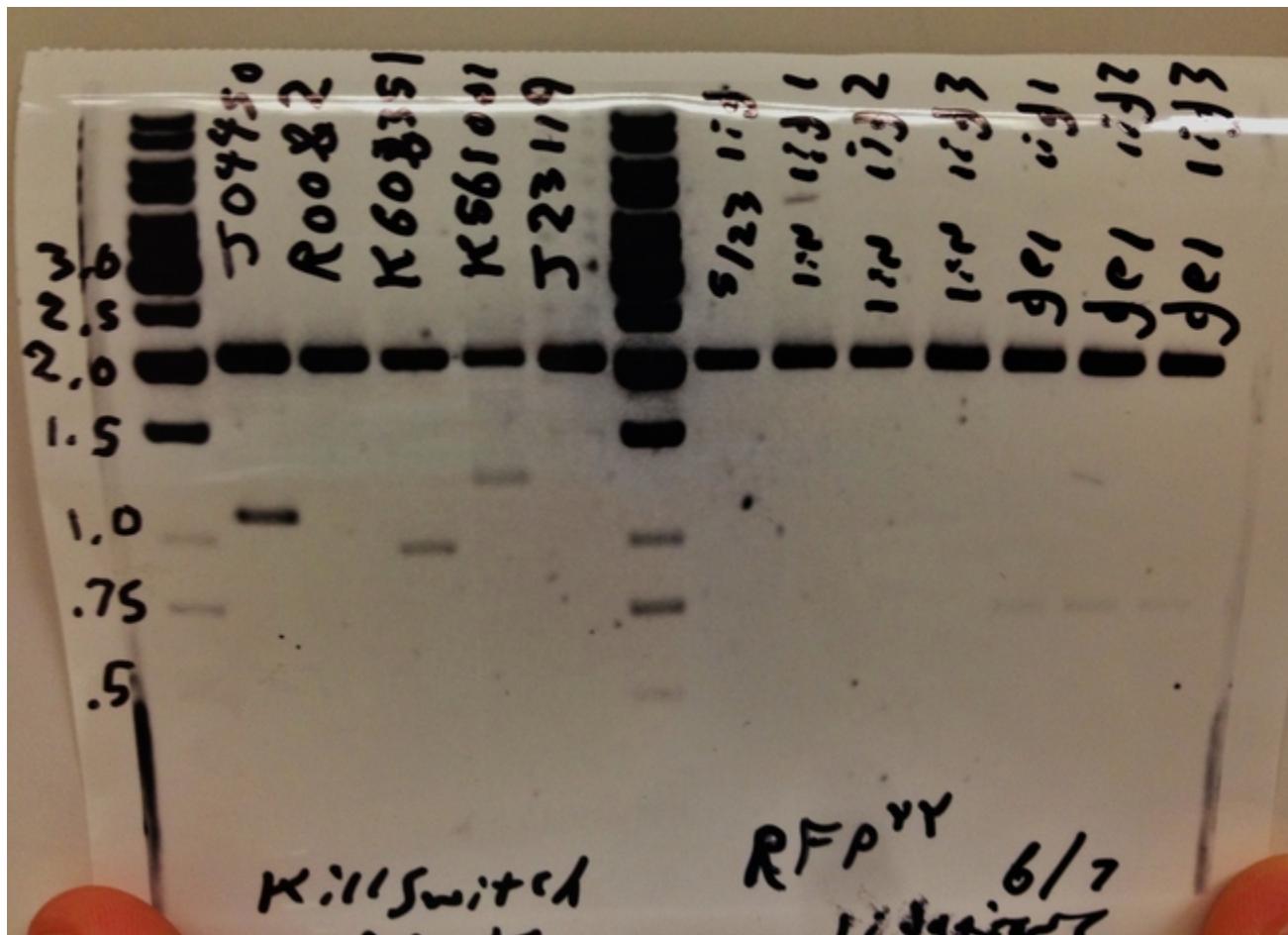
Id	Type	Name	Tube	Remarks
181				

Steps

#	Title	Timer
1	Make 5ml liquid culture of 3 colonies from linearized backbone ligation and three from gel extraction backbone ligation. Also make culture of re-done 5/23 ligation 2	00:00:00
2	Miniprep using Qiagen kit. Elute in 50 ul	00:00:00
3	Digest with EcoRI and PstI in NEBuffer 2.1. Run on 1.2% gel	00:00:00

Experiment Results

All three ligations from gel extracted backbone produce an insert at the correct size (713 bp). No ligations from PCR linearized backbone supplied by iGEM HQ produce inserts.



Experiment Attached Images

15-06-10_rfpYY_and_killswitch_digest.JPG

Experiment: **2015-06-13 Adding promoter**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Cloning YY Optimized RFP | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Samples & Reagents

Id	Type	Name	Tube	Remarks
231				

Steps

#	Title	Timer
1	Digest 5 ul of confirmed RFPyy gel lig 1 and 5 ul of E1010 RFP with 1 ul XbaI and 1 ul PstI in NEBuffer 2 (20 ul reaction). Digest 5 ul of K314100 promoter with 1ul SpeI and 1ul PstI in NEBuffer 2.1 (20 ul reaction). Incubate 1.0 hours at 37 degrees	00:00:00
2	Run all 20 ul of digest on .7% gel. Gel extract using Qiagen kit. Elute in 30ul and nanodrop (curve poor as expected, yield 6-18 ng/ul)	00:00:00
3	Ligation reactions: 13.5 ul of insert (either E1010 or RFPyy), 3.5 ul of backbone (K31400), 2 ul T4 ligase reaction buffer, 1 ul T4 DNA ligase. Incubate for 3 hours at room temperature, then store at 4 degrees	00:00:00
4	Transform each ligation (30 min ice, 50 sec 42 degrees, 5 min ice, 1 hour in 200 ul SOC)	00:00:00
5	After 36 hours of growth, make two 5ml liquid cultures per plate. All plates produce >100 colonies. E1010 with promoter are visibly red, but RFPyy with promoter has no visible color.	00:00:00
6	Miniprep samples using viogene kit. Elute in 50 ul. All [DNA]>200 ng/ul with good curve.	00:00:00
7	Send sample 1A of RFPyy + K31400 to Geneqiz for sequencing with VF2 primer	00:00:00

Milestone: **Revised GFP UV Test**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Testing the same hypothesis as the UV growth curve experiment (that mutated cells will cause population to lose fluorescence over time) with the following revisions: using low copy number backbone (pSB3K3), replating a much larger dilution (1:1.5), and measuring GFP fluorescence on the fluorometer

Experiment: **2015-06-01 Setting up plates (old protocol)**

**Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Revised GFP UV Test
| Owner: Jarrod Shilts**

Experiment Procedures

First procedure

Steps

#	Title																				
1	<p>Grow three 5ml cultures of constitutive promoter attached to BBa_E0240 GFP in pSB3K3 (with kanamycin selection) generator without a promoter</p>																				
2	<p>After 12 hours of growth, take OD600 measurements and dilute as necessary to equalize</p>																				
3	<p>Set up 96 well plate</p> <table border="1"> <thead> <tr> <th></th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>6</th> <th>7</th> <th>8</th> <th>9</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>100 ul GFP, with 0 UV irradiation, from culture A +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture A +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture A +150ul LB+Kan</td> <td>100 ul GFP, with 0 UV irradiation, from culture B +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture B +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture B +150ul LB+Kan</td> <td>100 ul GFP, with 0 UV irradiation, from culture C +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture C +150ul LB+Kan</td> <td>100 ul GFP, with 5000 J/M2 UV irradiation, from culture C +150ul LB+Kan</td> </tr> </tbody> </table>		1	2	3	4	5	6	7	8	9	A	100 ul GFP, with 0 UV irradiation, from culture A +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture A +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture A +150ul LB+Kan	100 ul GFP, with 0 UV irradiation, from culture B +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture B +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture B +150ul LB+Kan	100 ul GFP, with 0 UV irradiation, from culture C +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture C +150ul LB+Kan	100 ul GFP, with 5000 J/M2 UV irradiation, from culture C +150ul LB+Kan
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4	<p>Take initial measurement of absorption at 600 and fluorescence at excitation 485/15, emission 516/20. Wrap in parafilm and place in shaking incubator for 24 hours</p>																				

Samples & Reagents

Id	Type	Name	Tube	Remarks
81				

Experiment: **2015-06-01 - Setting up plates (new protocol)**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Revised GFP UV Test**
| Owner: **Jarrod Shilts**

Experiment Procedures

Setting up plates (new protocol)

Samples & Reagents

Id	Type	Name	Tube	Remarks
111				

Steps

#	Title	Timer														
1	Grow three cultures of I20260 (Promoter+GFP+pSB3K3) for 24 hours in 3ml liquid culture+Kan. Also grow San4 (no promoter+random insert) for 24 hours in 3ml liquid culture+CMR. Grow both from 5 ul of prior culture. After 24 hours, verify that both have equal OD readings	00:00:00														
2	Set up 96 well plate (for measurements only). Pipette 200 ul into the two wells with no UV exposure. Also make 3 ml liquid cultures with 200 ul of culture for those two. Then, expose the four liquid cultures to 5000 J/M2 of UV. Immediately pipette 200 ul of mixed culture into the remaining wells	00:00:00														
	<table border="1"> <thead> <tr> <th></th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>6</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>GFP with 0 J/ M2 UV. From culture A</td> <td>GFP with 5000 J/ M2 UV. From culture A</td> <td>GFP with 5000 J/ M2 UV. From culture B</td> <td>GFP with 5000 J/ M2 UV. From culture C</td> <td>San4 with 0 J/ M2 UV</td> <td>San4 with 5000 J/M2 UV</td> </tr> </tbody> </table>		1	2	3	4	5	6	A	GFP with 0 J/ M2 UV. From culture A	GFP with 5000 J/ M2 UV. From culture A	GFP with 5000 J/ M2 UV. From culture B	GFP with 5000 J/ M2 UV. From culture C	San4 with 0 J/ M2 UV	San4 with 5000 J/M2 UV	
	1	2	3	4	5	6										
A	GFP with 0 J/ M2 UV. From culture A	GFP with 5000 J/ M2 UV. From culture A	GFP with 5000 J/ M2 UV. From culture B	GFP with 5000 J/ M2 UV. From culture C	San4 with 0 J/ M2 UV	San4 with 5000 J/M2 UV										
3	With fluorometer, measure absorption at 600nm and fluorescence at excitation 485/15, emission 516/20.	00:00:00														
4	Make 3 ml liquid cultures (with appropriate antibiotic) for the four remaining samples. Add 200 ul of culture to each. Incubate all tubes on 225 RPM shaking incubator at 37 degrees	00:00:00														

Milestone: **Optimizing T4 PDG Assay**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Owner: **Jarrod Shilts**

Experiment: **2015-06-20 PCR Extraction**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Optimizing T4 PDG Assay** | Owner: **Jarrod Shilts**

Signed by Ophir Ospovat on July 01, 2015 at 10:29

Experiment Procedures

First procedure

Steps

#	Title	Timer
1	Use 2x Q5 master mix to PCR K314100+E1010 (lig 1A) and K314100+RFPyy (lig 1A). Use 1.2 ul of template diluted 1:100 (~3 ng) with VF2 and VR primers. Ta=66 degrees, extension time of 45 seconds. Two 50 ul reactions each	00:00:00
2	Run 3 ul of each of the four PCR products on pre-set 0.7% gel	00:00:00
3		00:00:00

Experiment: 2015-06-22 T4 PDG Reaction

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimizing T4 PDG Assay | Owner: Jarrod Shilts

Signed by Ophir Ospovat on June 26, 2015 at 03:20

Experiment Procedures

In vitro UV Dimer Assay

Steps

#	Title	Timer																				
1	To PCR tube, add 400 ng of PCR purified product (~3.5 ul) and bring up to 4 ul with water. Repeat with second set of tubes, adding 600 ng (~5 ul) and bring up to 6 ul with water. Make 4 tubes for 400 ng, and 4 for 600 ng (E1010 control, RFPyy control, E1010 UV, RFPyy UV)	00:00:00																				
2	Place PCR tubes on side in UV crosslinker box. Expose to 3000 J/cm ² of UV	00:00:00																				
3	To each tube, add 2 ul of 10x reaction buffer, 0.2 ul of 100x BSA, and either 8.8 ul or 11.8 ul water. Add 1 ul of enzyme at 10 units/ul per 200 ng (2 ul to 400 ng, 3ul to 600 ng).	00:00:00																				
4	Incubate reactions at 37 degrees for 20 minutes. Immediately after incubation, add SDS for a final concentration of 1% then store in cold room until alkaline gel prepared	00:00:00																				
5	Pour alkaline agarose gel at 1%. Expose to 10-15 seconds bursts in microwave until all dissolved. Mass afterward to ensure little volume was lost (add ~2ml extra before microwaving)	00:00:00																				
6	Cast gel and let solidify for 30 minutes. Then submerge in alkaline running buffer for one hour	00:00:00																				
7	Cool gel in cold room. Add 4 ul of alkaline gel loading dye to 20 ul of reaction. Place gel at room temperature and load all 24 ul	00:00:00																				
<table border="1"> <tr> <td>KB ladder</td> <td>400 ng E1010 no UV</td> <td>400 ng E1010 with UV</td> <td>400 ng RFPyy no UV</td> <td>400 ng RFPyy with UV</td> <td>100 bp ladder</td> <td>600 ng E1010 no UV</td> <td>600 ng RFPyy no UV</td> <td>600 ng E1010 with UV</td> <td>600 ng RFPyy with UV</td> <td>2-log ladder</td> </tr> </table>												KB ladder	400 ng E1010 no UV	400 ng E1010 with UV	400 ng RFPyy no UV	400 ng RFPyy with UV	100 bp ladder	600 ng E1010 no UV	600 ng RFPyy no UV	600 ng E1010 with UV	600 ng RFPyy with UV	2-log ladder
KB ladder	400 ng E1010 no UV	400 ng E1010 with UV	400 ng RFPyy no UV	400 ng RFPyy with UV	100 bp ladder	600 ng E1010 no UV	600 ng RFPyy no UV	600 ng E1010 with UV	600 ng RFPyy with UV	2-log ladder												

8	Run between 95-115 volts for one hour. After, Submerge gel in stopping solution for 30 minutes, then add 5 ul EtBr per 100 ul of stopping solution and incubate 30 minutes before imaging.	00:00:00
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Experiment Results

Gel dissolved when run at room temperature. Yellow color produced possibly by acidification of bromcresol green dye. Voltage used for running gel exceeded recommended limits once voltage corrected for by the length of the electrophoresis box (3 V/cm² on BioRad box is approximately 75 volts).

Experiment Conclusion

Running in cold could help prevent agarose dissolution at temperatures above 40 degrees. Experiment will be repeated in the cold.

Experiment: 2015-06-23 T4 PDG Reaction

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimizing T4 PDG Assay | Owner: Jarrod Shilts

Signed by Ophir Ospovat on June 26, 2015 at 03:20

This experiment is a duplicate of [2015-06-22 T4 PDG Reaction](#)

Experiment Procedures

In vitro UV Dimer Assay

Steps

#	Title	Timer											
1	To PCR tube, add 400 ng of PCR purified product (~3.5 µl) and bring up to 4 µl with water. Make 8 tubes of 400 ng ([E1010 control, RFPyy control, E1010 UV, RFPyy UV]x2).	00:00:00											
2	Place PCR tubes on side in UV crosslinker box. Expose 4 tubes to 1000 J/cm2 of UV and 4 tubes to 5000 J/cm2 of UV.	00:00:00											
3	To each tube, add 2 µl of 10x reaction buffer, 0.2 µl of 100x BSA, and 11.8 µl water. Add 1 µl of enzyme at 10 units/µl per 200 ng (2 µl for 400 ng).	00:00:00											
4	Incubate reactions at 37°C for 25 minutes. Immediately after incubation, place tubes in thermocycler for 5 minutes at 70°C and then 3 minutes at 4°C	00:00:00											
5	Pour alkaline agarose gel at 1%. Expose to 10-15 seconds bursts in microwave until all dissolved. Mass afterwards to ensure little volume was lost (add ~2ml extra before microwaving).	00:00:00											
6	Cast gel and let solidify for 30 minutes then submerge in alkaline running buffer for one hour.	00:00:00											
7	Cool gel in cold room. Add 4 µl of alkaline gel loading dye to 20 µl of reaction and load all 24 µl.	00:00:00											
	<table border="1"> <tr> <td>100 bp ladder</td> <td>400 ng E1010 no UV</td> <td>400 ng E1010 with 1000 UV</td> <td>400 ng RFPyy no UV</td> <td>400 ng RFPyy with 1000 UV</td> <td>2-log ladder</td> <td>400 ng E1010 no UV</td> <td>400 ng RFPyy no UV</td> <td>400 ng E1010 with 3000 UV</td> <td>400 ng RFPyy with 3000 UV</td> <td>KBladder</td> </tr> </table>	100 bp ladder	400 ng E1010 no UV	400 ng E1010 with 1000 UV	400 ng RFPyy no UV	400 ng RFPyy with 1000 UV	2-log ladder	400 ng E1010 no UV	400 ng RFPyy no UV	400 ng E1010 with 3000 UV	400 ng RFPyy with 3000 UV	KBladder	
100 bp ladder	400 ng E1010 no UV	400 ng E1010 with 1000 UV	400 ng RFPyy no UV	400 ng RFPyy with 1000 UV	2-log ladder	400 ng E1010 no UV	400 ng RFPyy no UV	400 ng E1010 with 3000 UV	400 ng RFPyy with 3000 UV	KBladder			

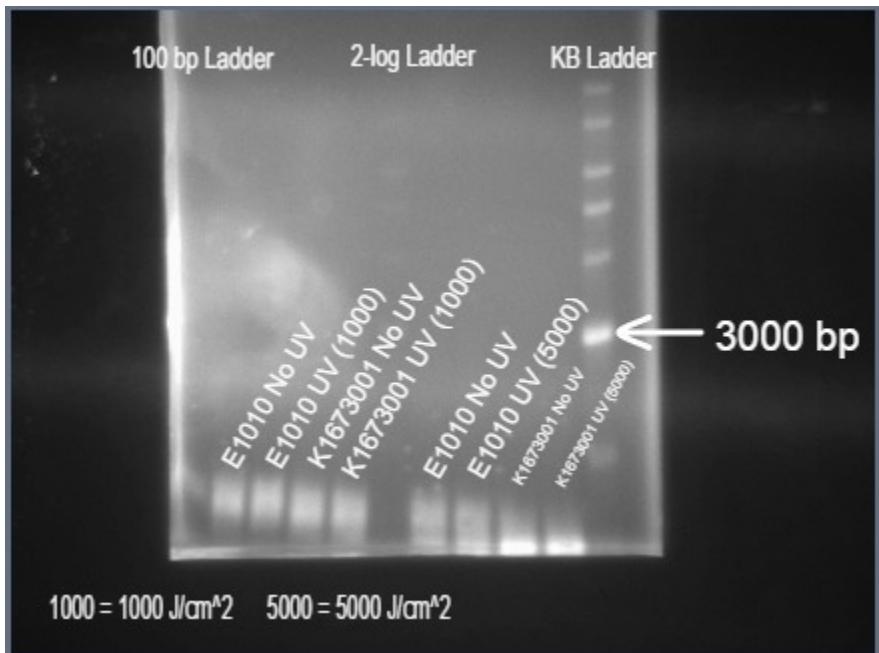
8	Run at 75 V for 3 hours in a cold room. Afterwards, submerge gel in stopping solution for 30 minutes, then add 5 µl/100 ml EtBr to the stopping solution and incubate 30 minutes before imaging.	00:00:00
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Procedure Notes:

Gel was incubating with buffer in cold room for 5 hours before running.

Experiment Results

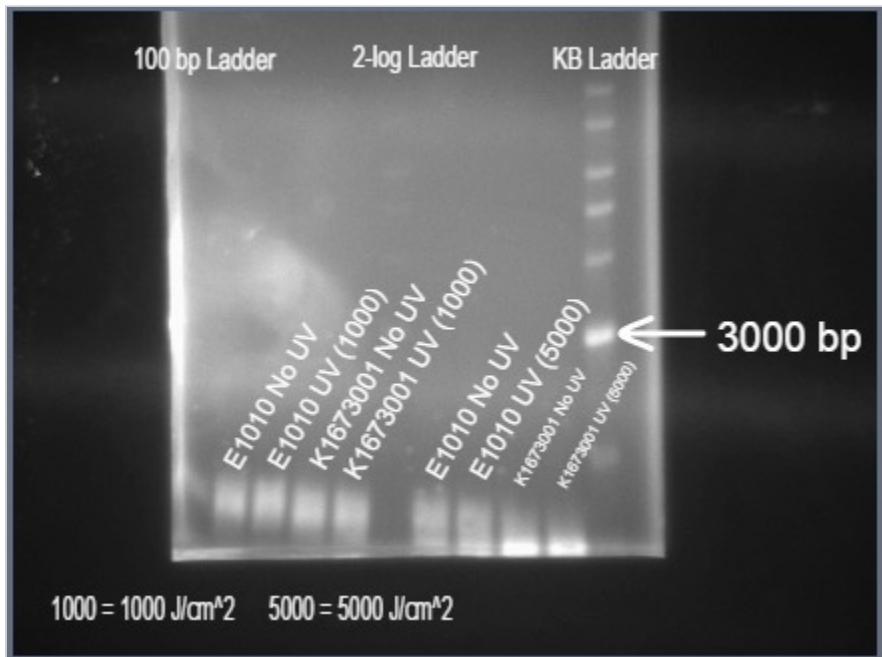
Out of all the standards, only the KB Ladder was visible. The samples at 1000 J/cm² were slightly visible but those at 5000 J/cm² were effectively not visible at all due to being run off the gel. The crookedness of the gel can be attributed to a non-parallel electrode.



Experiment Conclusion

Assay will be repeated with changed parameters for UV exposure, enzyme incubation time, and gel running time. In addition, two controls of undigested DNA will be added to asses the characteristics of the alkaline gel. No quantitative conclusion can be drawn from this gel due to the loss of sample from the gel.

Experiment Attached Images



23-06-15_In_vitro_PDG.jpg

Experiment: 2015-06-26 PCR Extraction 2

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimizing T4 PDG Assay | Owner: Ophir Ospovat

Signed by Ophir Ospovat on June 26, 2015 at 03:20

Purpose: Amplify specific segment of DNA

Ingredients

- Q5 Hot Start High-Fidelity 2X Master Mix
- 5' Forward Primer
- 3' Reverse Primer
- Template DNA
- ddH₂O

Experiment Procedures

Primer Dilution

Steps

#	Title	Timer
1	Centrifuge tube to collect DNA pellet at the bottom of the tube	00:00:00
2	Add 10x μ l ddH ₂ O to DNA in primer tube to create a 100 μ M primer stock solution (x = nanomolar quantity)	00:00:00
3	Use pipetting to resuspend DNA	00:00:00
4	Add 90 μ l of ddH ₂ O to a microcentrifuge tube labeled with primer name	00:00:00
5	Take 10 μ l of primer stock solution for 100 μ l total volume and 10 μ M primer working solution	00:00:00

Preparation

Steps

#	Title	Timer
1	Prepare master solution by combining the following in a PCR tube:	00:00:00

Ingredient	Volume (1x)	Volume (2x)
Q5 2x Master Mix	12.5 µl	25 µl
10µM 5' Forward Primer	1.25 µl	2.5 µl
10µM 3' Forward Primer	1.25 µl	2.5 µl
Template DNA*	variable	variable
ddH ₂ O	variable	variable
Total	25 µl	50 µl

*Plasmid (1 pg–1 ng), Genomic (1 ng–1 µg) per 50 µl reaction

Running PCR

Steps

#	Title	Timer
1	Fill each properly labeled PCR tube with master mix (Q5 Master Mix, Template, Primers, Water)	00:00:00
2	Place PCR tubes in Thermocycler and select the appropriate program	00:00:00
3	Edit the program for the correct extension time (30 sec/kbp) and annealing temperature (usually 66° C)	00:00:00
4	After PCR cycle completion, store at 4° C and/or run the PCR product on the gel	00:00:00

*Use [NEB Tm Calculator](#) to determine correct annealing temperature

Experiment Notes

Template DNA: BBa_K1673001/BBa_K1673002

Amplicon: K314100+RFP^{YY}/K314100+E1010

Forward Primer: VF2

Reverse Primer: VR

Reactions: 4x50 µl/ 4x50 µl

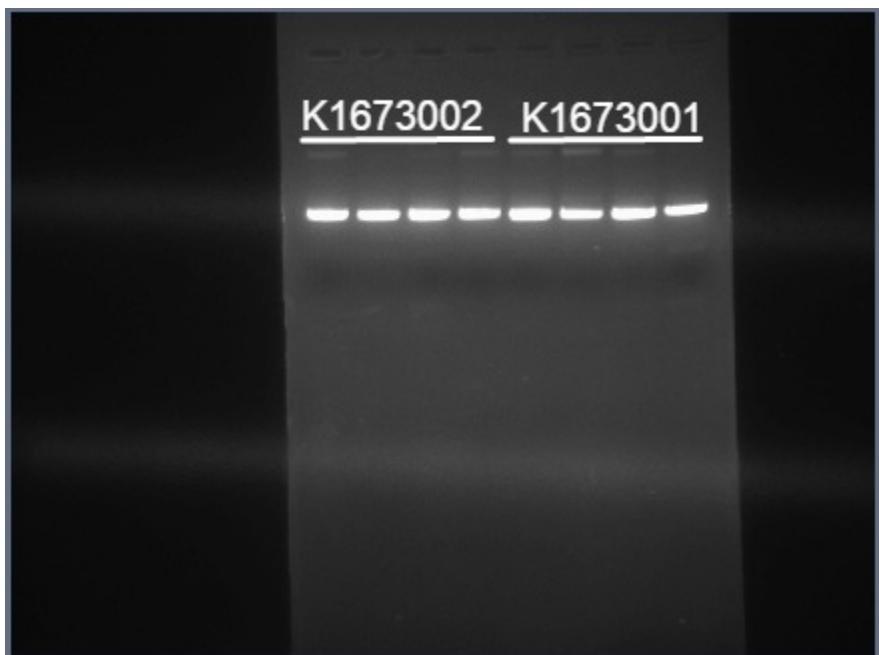
T_a = 66° C

Extension Time: 60 seconds

Cycles: 35

Experiment Results

Running 1 µl of PCR reaction on gel indicates that the PCR worked successfully for both the wild type and optimized RFP with constitutive promoter.



The reactions were respectively combined and purified using a Qiaquick PCR Purification Kit with an elution volume of 50 µl of ddH₂O.

K314100+RFP^{YY} - 368.1 ng/μl

K314100+E1010 - 419.0 ng/μl

Experiment Conclusion

DNA will be used in future with in vitro PDG assays.

Experiment Attached Images

PCR_for_PDG_Assay.jpg

Linked Resources

- Easy Polymerase Chain Reaction (NEB) (Protocol)

Experiment: 2015-06-26 T4 PDG Reaction

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimizing T4 PDG Assay | Owner: Ophir Ospovat

Signed by Ophir Ospovat on June 28, 2015 at 02:10

This experiment is a duplicate of [2015-06-23 T4 PDG Reaction](#)

Experiment Procedures

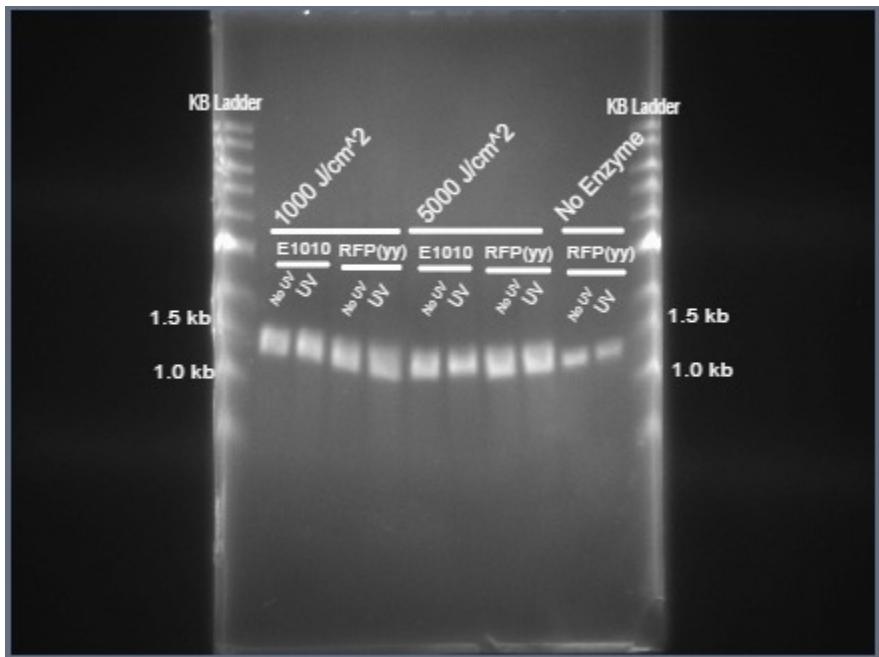
In vitro UV Dimer Assay

Steps

#	Title	Timer
1	To PCR tube, add 400 ng of PCR purified product (~1.0 ul) and bring up to 4 ul with water. Make 10 tubes of 400 ng ([E1010 control, RFPyy control, E1010 UV, RFPyy UV]x2+E1010x2).	00:00:00
2	Place PCR tubes on side in UV crosslinker box. Expose 2 tubes to 1000 J/cm ² of UV and 3 tubes to 5000 J/cm ² of UV.	00:00:00
3	To each tube, add 2 ul of 10x reaction buffer, 0.2 ul of 100x BSA, and 11.8 ul water. Add 1 ul of enzyme at 10 units/ul per 200 ng (2 ul to 400 ng).	00:00:00
4	Incubate reactions at 37°C for 30 minutes. Immediately after incubation, place tubes in thermocycler for 5 minutes at 70°C and then 3 minutes at 4°C	00:00:00
5	Pour alkaline agarose gel at 1%. Expose to 10-15 seconds bursts in microwave until all dissolved. Mass afterward to ensure little volume was lost (add ~2ml extra before microwaving).	00:00:00
6	Cast gel and let solidify for 30 minutes then submerge in alkaline running buffer for one hour.	00:00:00
7	Cool gel in cold room. Add 4 ul of alkaline gel loading dye to 20 ul of reaction and load all 24 ul.	00:00:00
8	Run at 75 volts for 2 hours on an ice tray in a cold room. Afterwards, submerge gel in 60 ml stopping solution for 30 minutes, then add 5 ul/100 ml EtBr to the stopping solution and incubate 30 minutes before imaging.	00:00:00

Experiment Results

Gel was successfully run for the first time. Ladder showed up well with all bands visible; the PCR products ran between 1.2 and 1.5 kb. The no enzyme controls correctly show less smearing as no digestion took place and they are also at the right size (~1.3 kb). For both levels of UV irradiation, the RFP^{YY} bands look thicker than the E1010 bands and the No UV versus UV bands look identical in all samples pending further analysis.



Experiment Conclusion

Pending Analysis. Experiment will be repeated with whole plasmids in the future to determine if the enzyme acts differently on DNA fragments than as on plasmids.

Experiment Attached Images

26-06-15_In_vitro_PDG.jpg

Experiment: 2015-06-28 PDG Nicked Plasmid

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimizing T4 PDG Assay | Owner: Jarrod Shilts

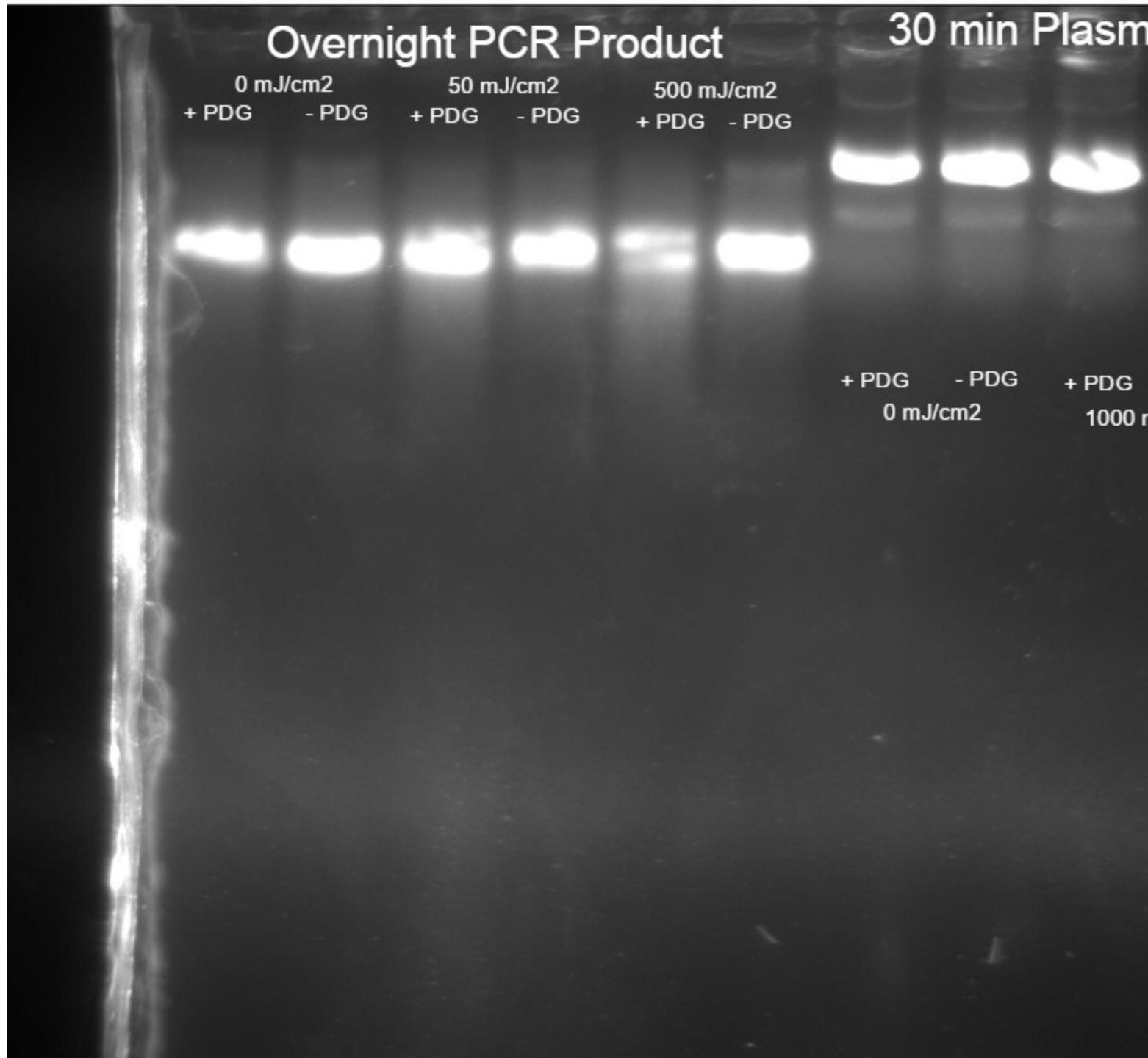
Experiment Procedures

New Procedure

Steps

#	Title	Timer														
1	Add 1ul of San4 Miniprep (220 ng) to 4 ul of water. Irradiate samples in UV crosslinker box (see below)	00:00:00														
	<table border="1"><tr><td>UV Exposure (mJ/cm2 = x/10 box reading)</td><td>0</td><td>0</td><td>30</td><td>30</td><td>300</td><td>300</td></tr><tr><td>PDG enzyme added</td><td>-</td><td>+</td><td>-</td><td>+</td><td>-</td><td>+</td></tr></table>	UV Exposure (mJ/cm2 = x/10 box reading)	0	0	30	30	300	300	PDG enzyme added	-	+	-	+	-	+	
UV Exposure (mJ/cm2 = x/10 box reading)	0	0	30	30	300	300										
PDG enzyme added	-	+	-	+	-	+										
2	Make master mix of 72 ul water, 12 ul 10x PDG buffer, and 1.2 ul 100x BSA. Add 14.1 ul of mix to each reaction tube. Add 1.1 ul of PDG or 1.1 ul of water depending on the condition	00:00:00														
3	Incubate 30 minutes at 37 degrees. Load all 20 ul of reaction on 1% agarose gel	00:00:00														

Experiment Results



Experiment: **2015-07-01 Overnight PDG incubation**

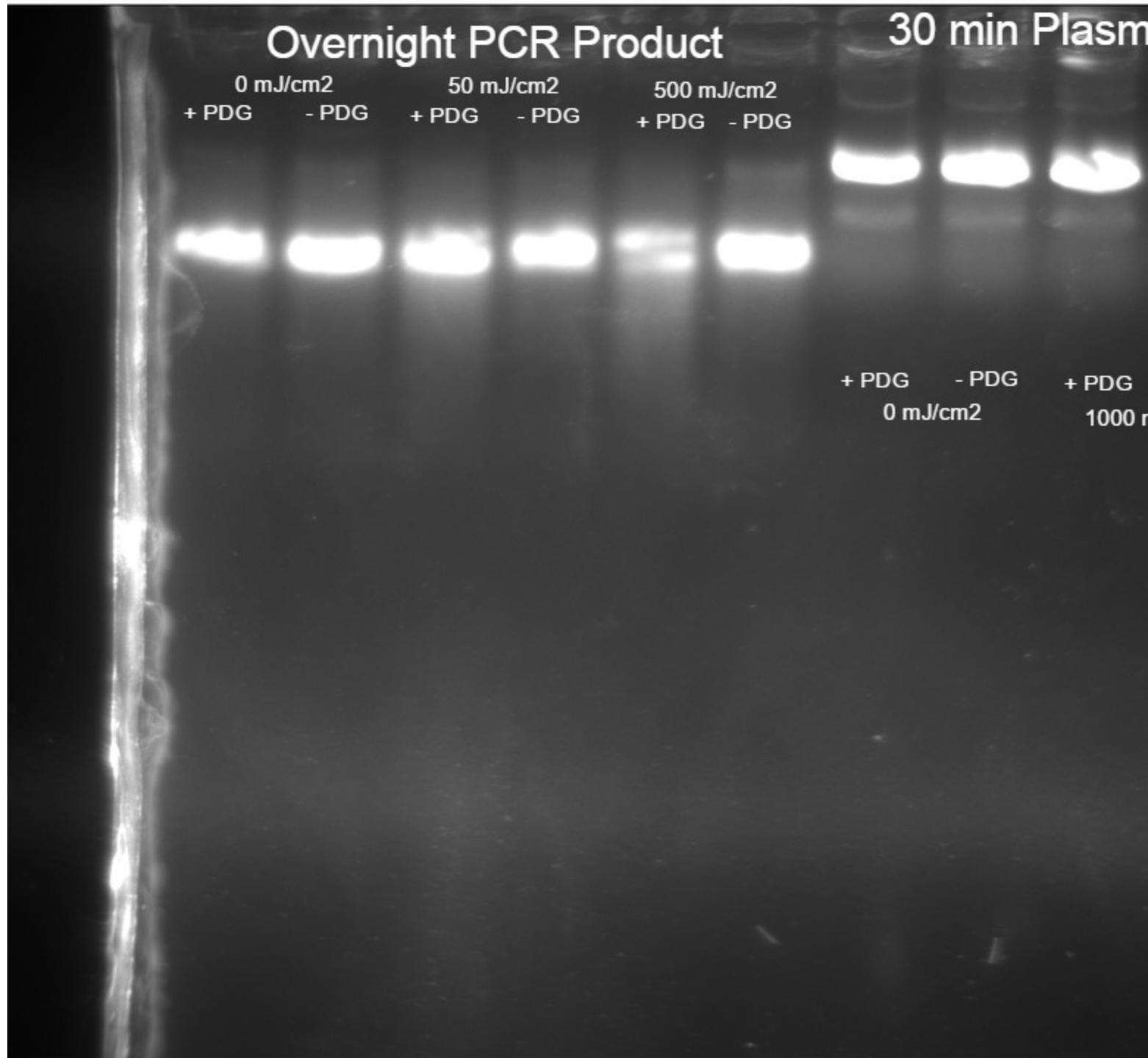
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 200 ng of PCR purified E1010 (0.45 ul) up to total volume of 5 ul in PCR tube. Expose two tubes to UV irradiation at 0 mJ/cm2, two at 50 mJ/cm2, and two at 500 mJ/cm2 (note: prior units used were off by factor of ten eg setting 5000 on machine = 500 mJ/cm2)	00:00:00
2	Set up 20 ul reaction for each tube, with 1 ul T4 PDG (10 units per 200 ng), 1x T4 buffer, and 1x BSA. Place at 37 degrees for 16 hours. Stop reaction by placing tubes at -20 degrees.	00:00:00
3	For comparison, make 4 reactions under the above protocol using San4 as DNA template. Expose to 0 UV and 100 mJ/cm2. Set up 20 ul reaction and incubate for 30 minutes at 37 degrees	00:00:00
4	Cast alkaline gel by dissolving 0.5g agarose in 47 ml of water. Let cool to 50 degrees, then add 5 ml of 10x alkaline gel buffer (made from 10 ml of 10 N NaOH and 4 ml of 0.5 M EDTA (pH 8.0) in 200 ml water). Cast in cold room, then submerge in 1x alkaline gel buffer at 4 degrees.	00:00:00
5	Add 1 ul of phenol to each reaction to separate enzyme from DNA. Then add 4.5 ul of 6x alkaline loading buffer. Load all 24 ul of reaction and run at 50 volts for 2 hours. Place glass on top of gel to prevent displacement	00:00:00
6	Soak in stopping solution 40 min, then switch solution to 50 ml TAE and add 5 ul of Diamond Nucleic Acid Dye. Incubate 20 min in dark at room temp	00:00:00

Experiment Results



Experiment Attached Images

7-1-15_pdg_overnight.jpg

Milestone: **Bulky DNA Lesion PCR**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: **2015-07-06 UV irradiated PCR**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Bulky DNA Lesion PCR | Owner: Jarrod Shilts

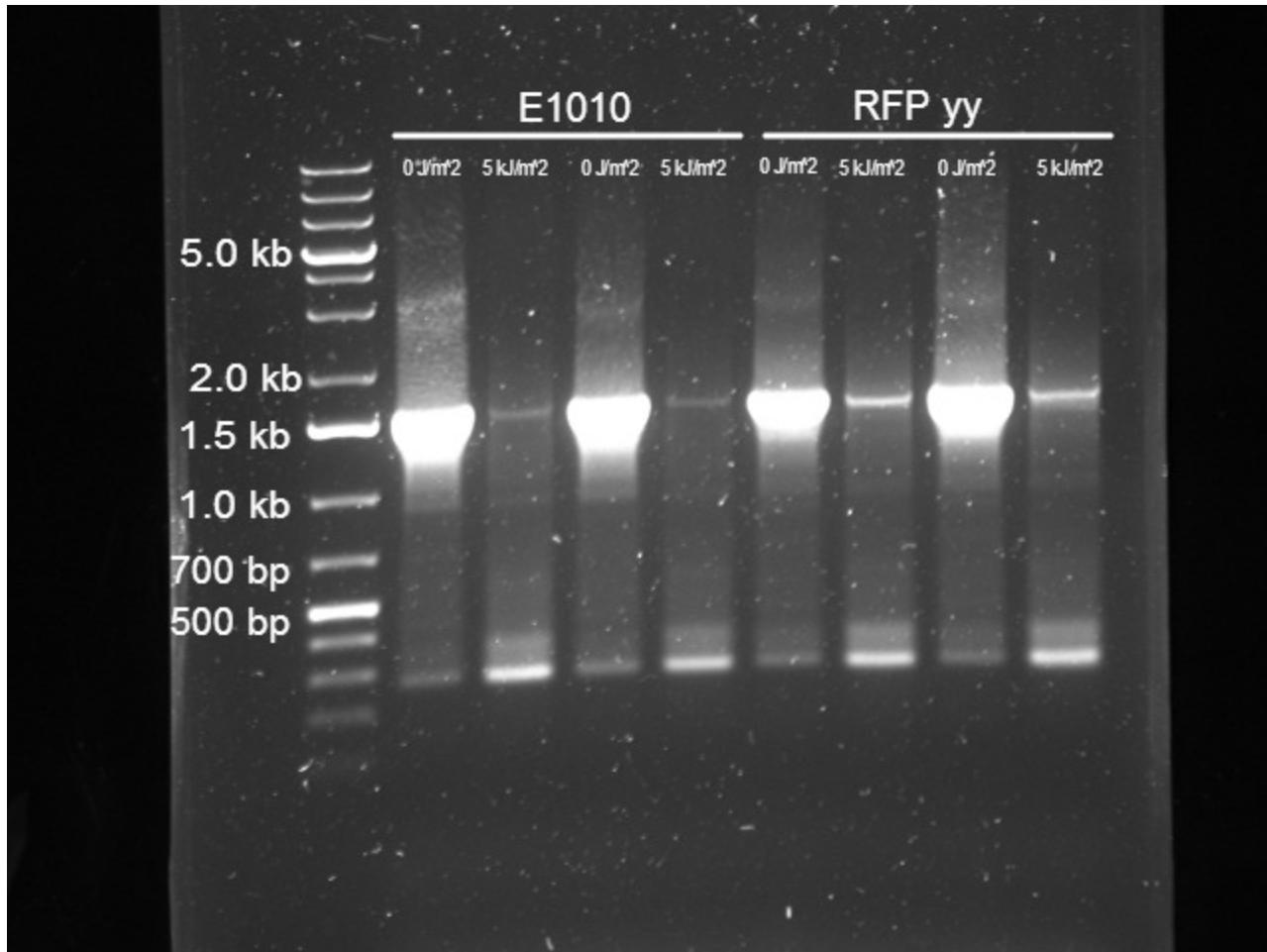
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 1000 ng of PCR purified K314100+E1010 or K314100+RFPyy to PCR tube. Irradiate at 5000 J/m ² or no irradiation. Keep tubes covered afterward	00:00:00
2	Set up 25 ul Platinum Taq PCR reaction. Add 100 ng of DNA sample to each tube. Run 60 sec 94 deg, 30 cycles 30 sec 94 deg 30 sec 55 deg 60 sec 72 deg, then 180 sec 72 deg,	00:00:00
3	Run on 1% standard agarose gel	00:00:00

Experiment Results



Experiment Conclusion

Visibly apparent improvement in the amount of PCR product at ~1.5 kb under engineered RFP sequence compared with control

Experiment: **2015-07-07 PDG PCR**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Bulky DNA Lesion PCR** | Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Irradiate E1010 and RFPyy with either 0 or 5000 J/m ² of UV	00:00:00
2	Incubate 600 ng of DNA in a 20 ul T4 PDG reaction with 1.5 ul of enzyme. Incubate one hour at 37 degrees.	00:00:00
3	Add either 1.5 ul or 0.5 ul of PDG reaction to 25 ul PCR mix with Greenbio Taq. PCR cycle of 30 sec 95 deg, 30 cycles 30 sec 95 deg, 30 sec 55 deg, 60 sec 68 deg, then 300 sec 68 deg.	00:00:00
4	Run on 1.2 % agarose gel	00:00:00

Experiment Results

Experiment Conclusion

No apparent difference observed. Variation in results depending strongly on amount of DNA used as template for PCR.

Experiment Attached Images

15-07-06_uv_pcr.tif

Experiment: **2015-07-08 UV PCR**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Bulky DNA Lesion PCR | Owner: Jarrod Shilts

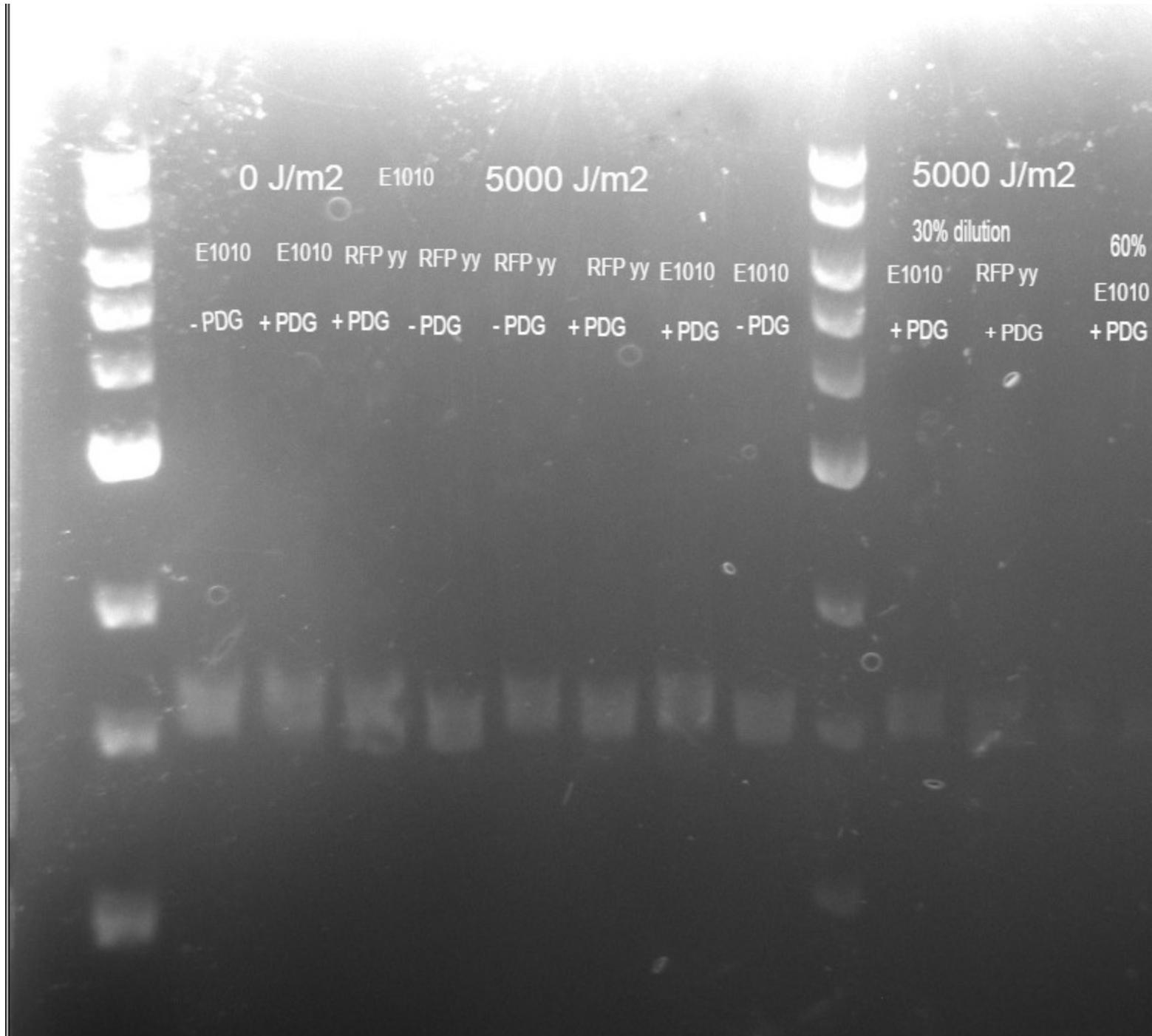
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Dilute K314100+E1010 and K314100+RFPyy PCR purified product to precisely 10 ng/ul. Add 5 ul of diluted PCR product to PCR tube and irradiate at 0 or 5000 J/m2	00:00:00
2	Incubate in a 10 ul T4 PDG reaction, with 0.2 ul of enzyme or 0.2 ul of water added per reaction. Incubate 30 min at 37 degrees. Left at room temp for 2 hours after incubation	00:00:00
3	25 ul Taq PCR reaction with VF2 and VR primers. 1 ul (5 ng) of DNA used as template for all reactions. Store at 4 degrees overnight	00:00:00
4	Run on 1 % agarose gel	00:00:00

Experiment Results



Experiment Attached Images

15-07-09.jpg

Milestone: RFPyy Expression Tests

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: 2015-06-20 Heterogeneity in RFP expression

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFPyy Expression Tests | Owner: Jarrod Shilts

Experiment Procedures

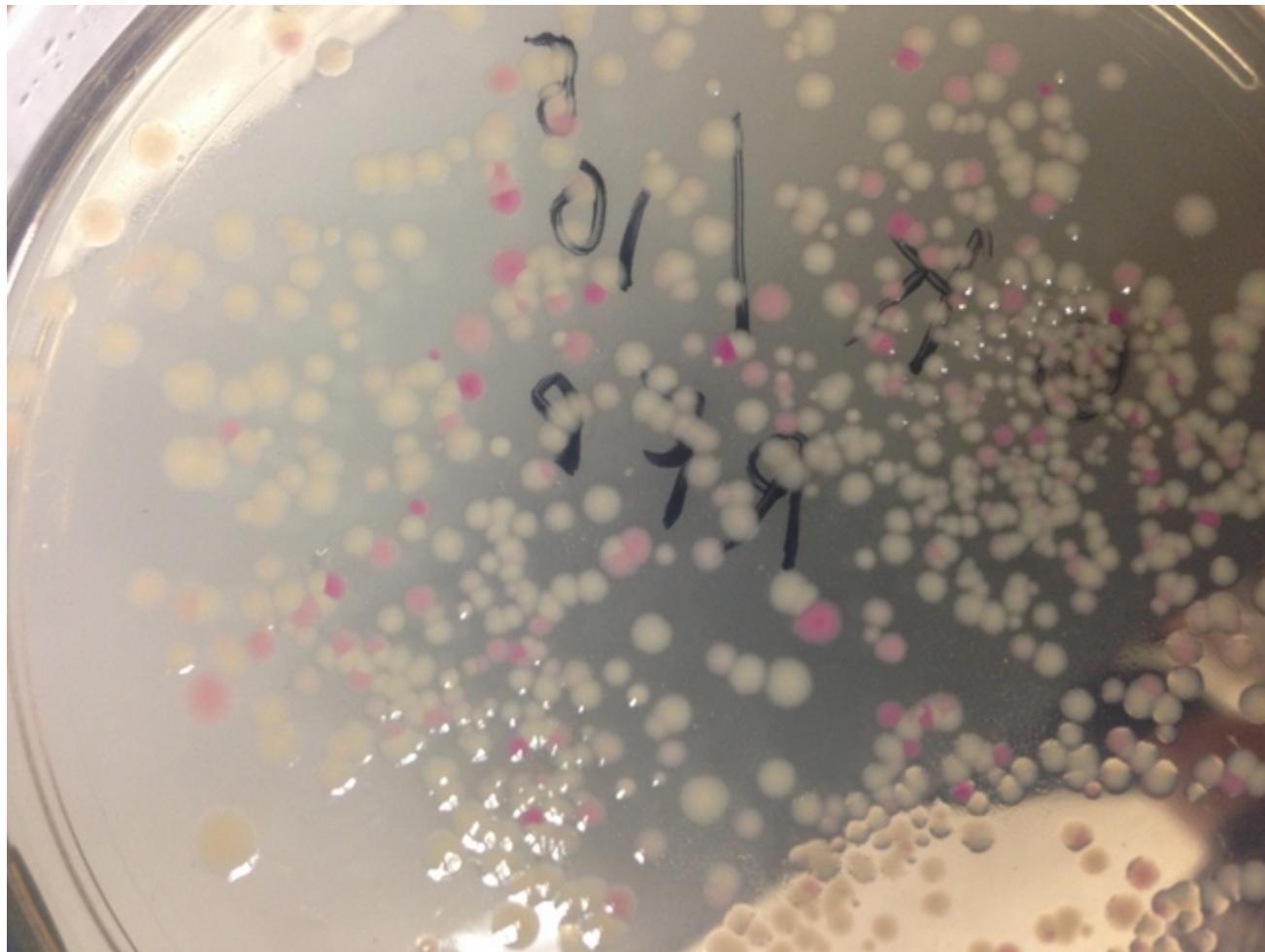
New Procedure

Steps

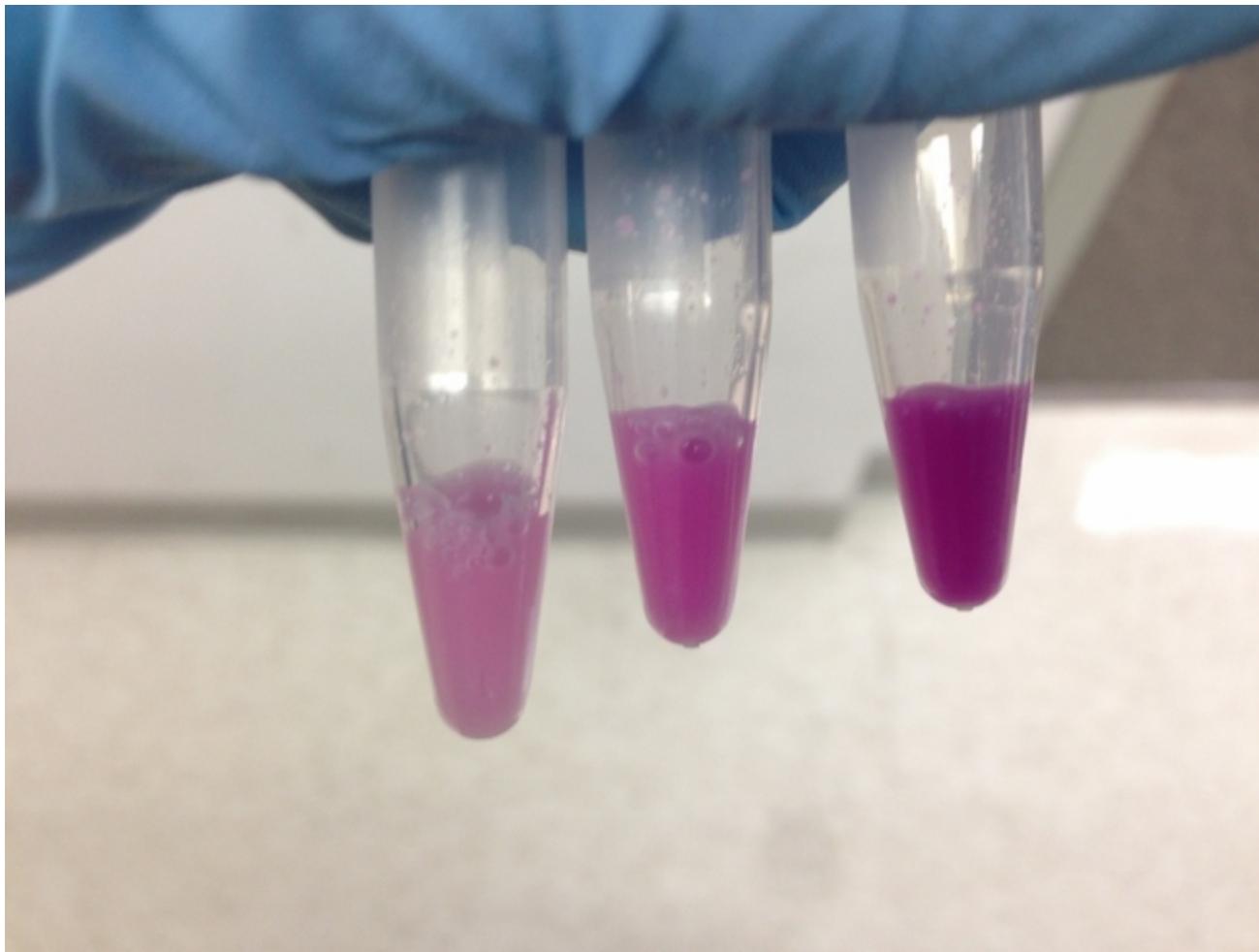
#	Title	Timer
1	Plate samples from second RFP expression test with no K395 added or 10^0 K395 added.	00:00:00
2	Image plates for differences in intensity. Grow liquid cultures of three colonies with no visible RFP expression, and three with dimmer than control (brightest spots equal to original J04450 intensity) RFP expression.	00:00:00
3	Miniprep cultures. Send samples for sequencing	00:00:00

Experiment Results

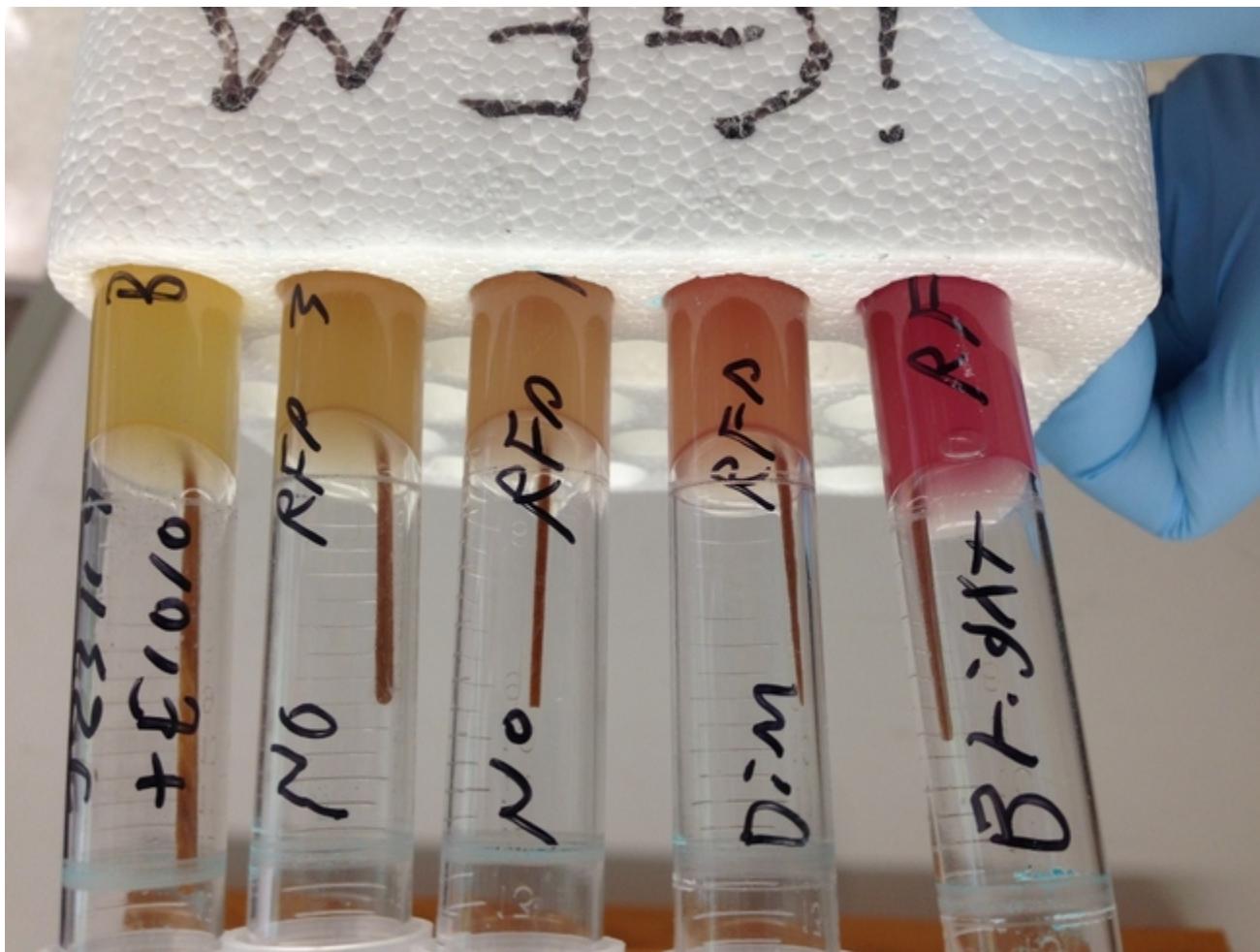
Example plate showing variable RFP expression



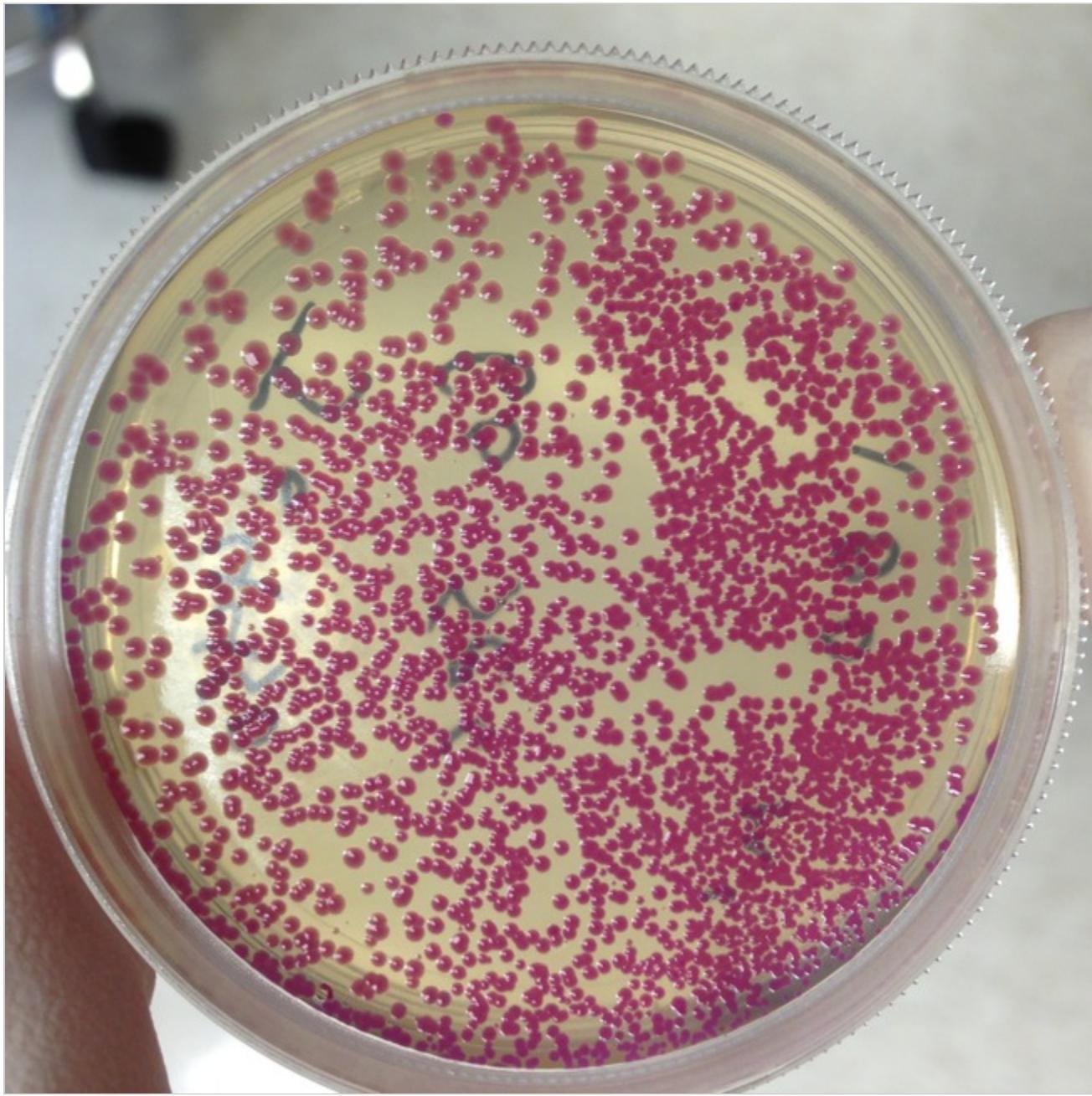
Visible RFP intensity of lysed cells (L->R no visible RFP on colony, dim, bright)



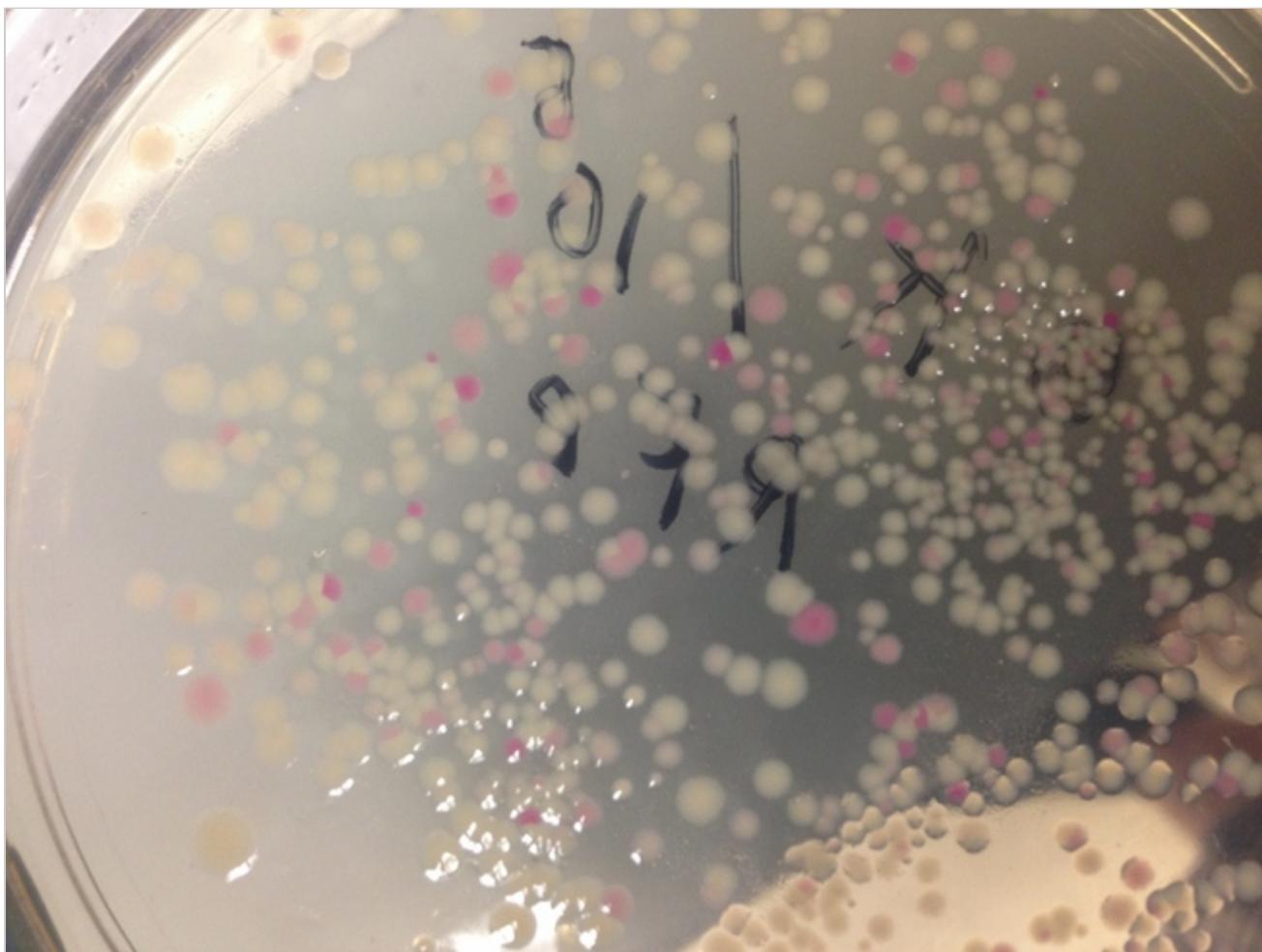
Culture tubes with variable expression



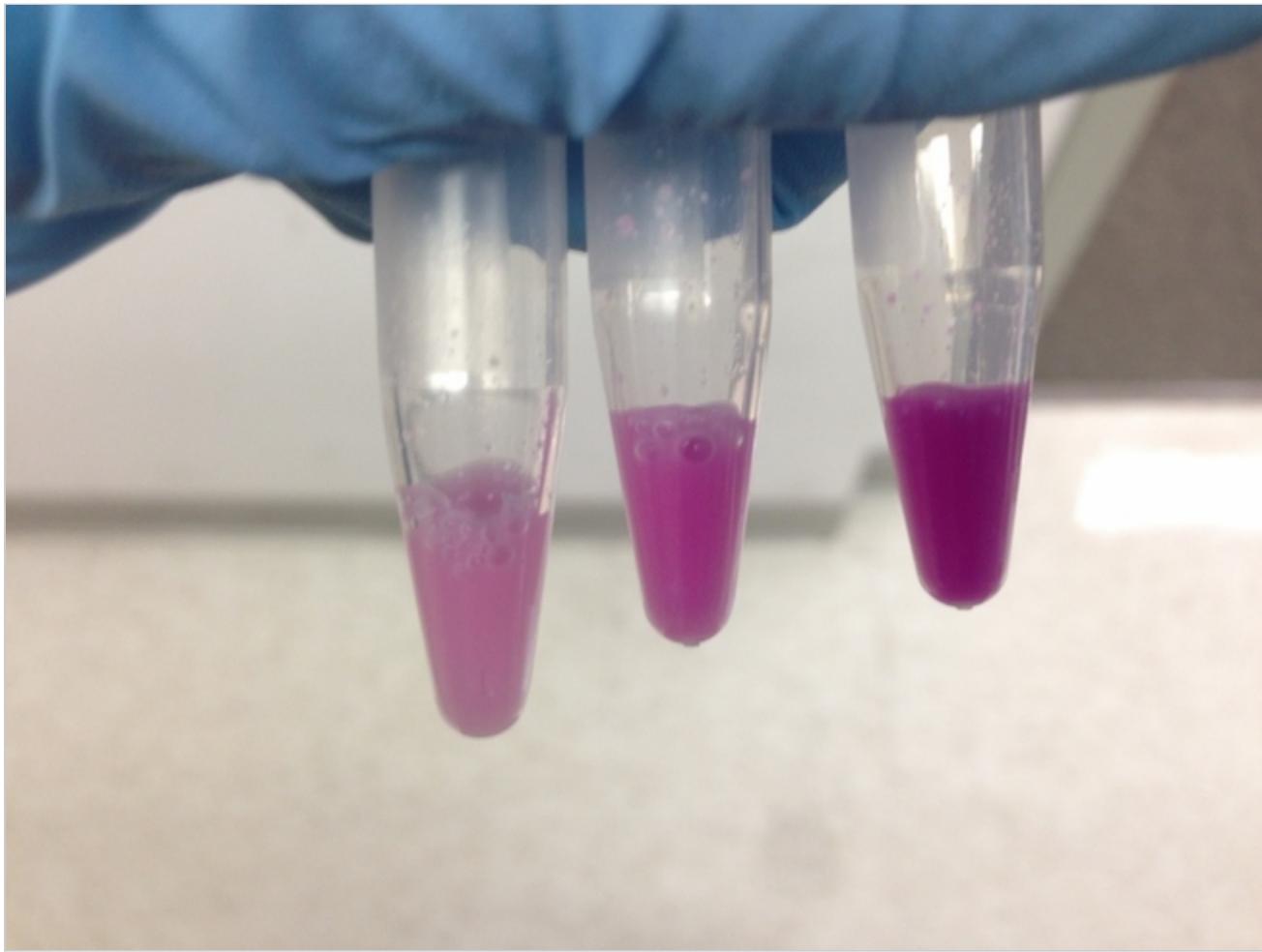
Experiment Attached Images



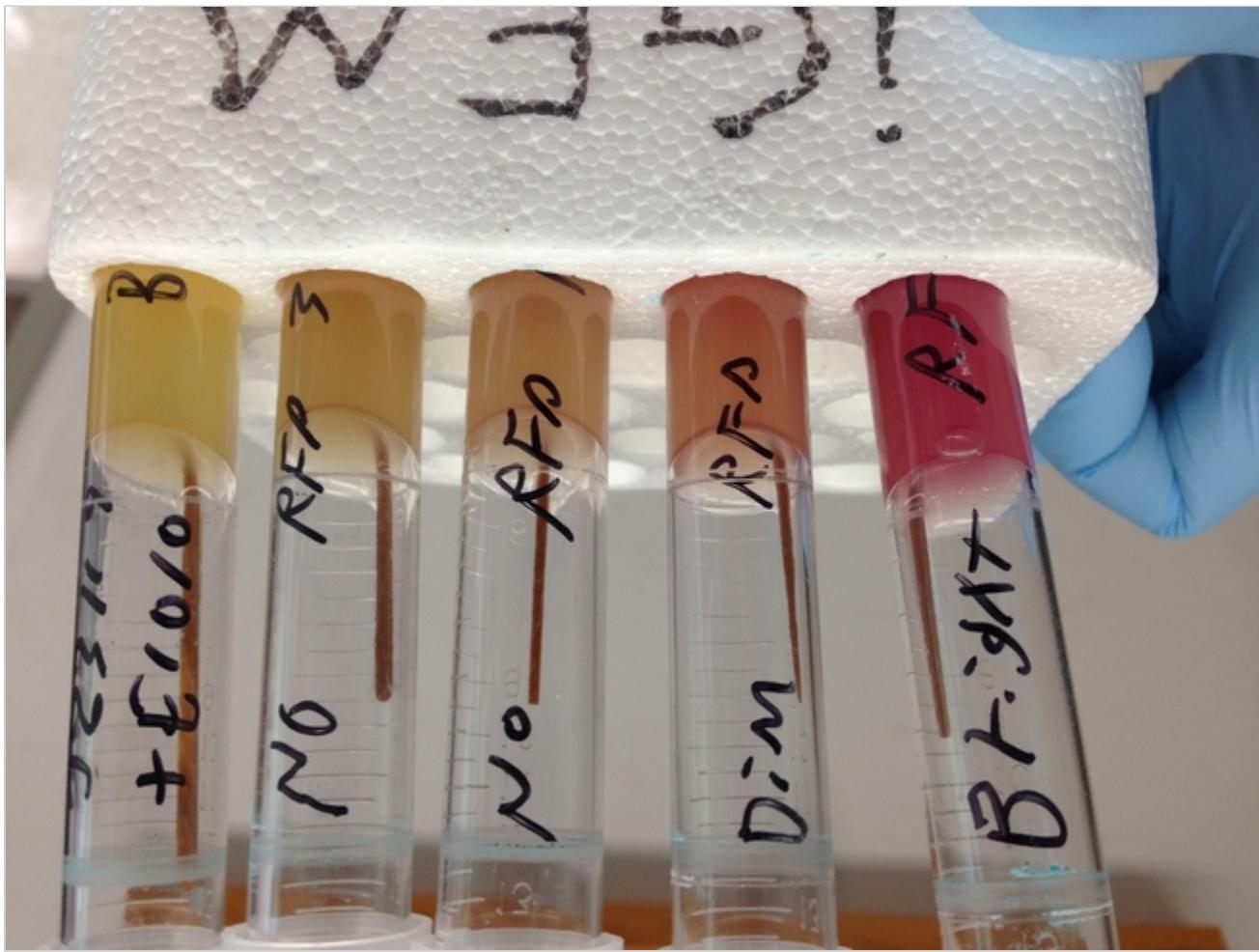
J04450.JPG



Two_week_RFP_variability_(768x1024).jpg



Miniprep_lysis_RFP_variability_(1024x768).jpg



Two_week_culture_RFP_variation_(1024x768).jpg

Experiment: 2015-06-27 Making Competent BL21 Cells

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFPyy Expression Tests | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Steps

#	Title	Timer
1	Grow 5 ml culture of LB with thawed DH5 alpha cells or thawed BL21 cells. Incubate for 24 hours at 37 degrees	00:00:00
2	Add 100 ml of LB to autoclaved 250 ml flask. Add 1 ml of starter culture and place in shaking incubator.	00:00:00
3	Using 96 well plate, measure OD at 30 minute intervals until reading is 0.4-0.5. For BL21 cells, OD of 0.404 reached after 3 hours.	00:00:00
4	Divide cell culture into two 50 ml Falcon tubes. Centrifuge 3000 G for 15 minutes at 4 degrees. Remove supernatant. In cold room, resuspend cells by pipette in 1 ml of ice cold 100 mM MgCl ₂ for both tubes. Then add 14 ml more 100 mM MgCl ₂ each. Vortex briefly.	00:00:00
5	Incubate cells in MgCl ₂ on ice for 5 minutes. Centrifuge 3000 G for 15 minutes at 4 degrees	00:00:00
6	Remove supernatant. In cold room, resuspend cells by pipette in 1 ml of ice cold 100 mM CaCl ₂ for both tubes. Then add 14 ml more 100 mM CaCl ₂ each.	00:00:00
7	Incubate cells in CaCl ₂ on ice for 45 minutes. Centrifuge 3000 G for 15 minutes at 4 degrees	00:00:00
8	Remove supernatant. In cold room, resuspend cells in 5ml of 100 mM CaCl ₂ and 30 % glycerol.	00:00:00
9	Place 100 eppendorf tubes, pre-chilled to -80 degrees, in crushed dry ice. Aliquot 50 ul of competent cells into each tube. Store at -80 degrees	00:00:00

Experiment Results

Experiment: **2015-06-28 Testing Competence and RFP expression**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **RFPyy Expression Tests** | Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

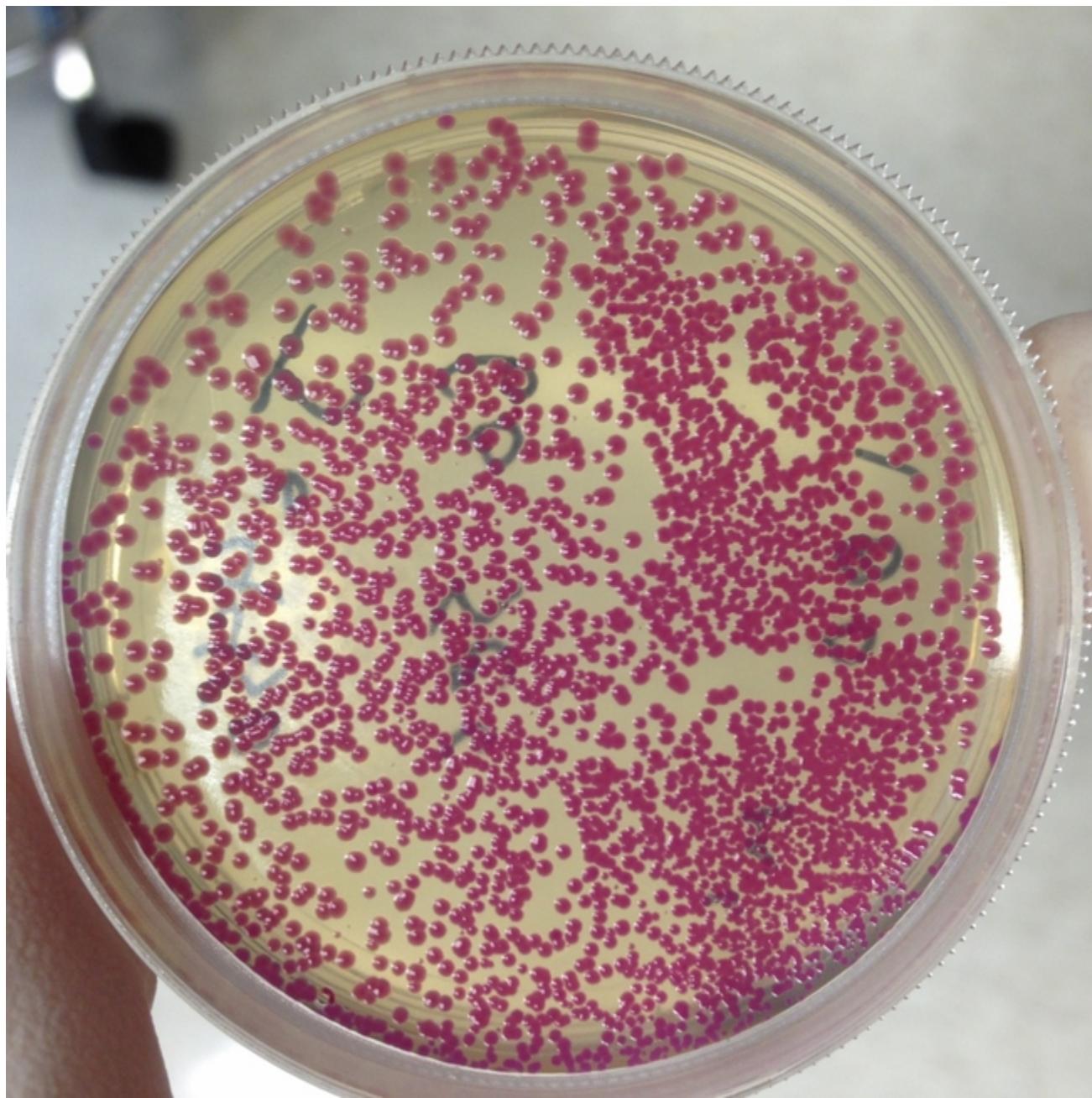
#	Title							
1	Thaw BL21 aliquots on ice for approximately 5-10 minutes.							
		Amount DNA Added:	50 pg of iGEM transformation kit J04450	50 pg of iGEM transformation kit J04450	50 pg of iGEM transformation kit J04450	100 ng of J04450 miniprep	100 ng of J04450 miniprep	200ng K314+ RFPyy
		Heat Shock Time:	10 seconds	30 seconds	45 seconds	20 seconds	60 seconds	10 seconds
		Number Transformants:						200ng K314+RFPyy
2	Incubate cells on ice for 30 minutes (timed from when DNA added to first tube)							
3	Heat shock at 42 degrees for the times listed above. Place back on ice for 5 minutes							
4	Add 450 ul of room-temperature SOC media. Outgrowth in shaking incubator for 1 hour.							

Experiment Results

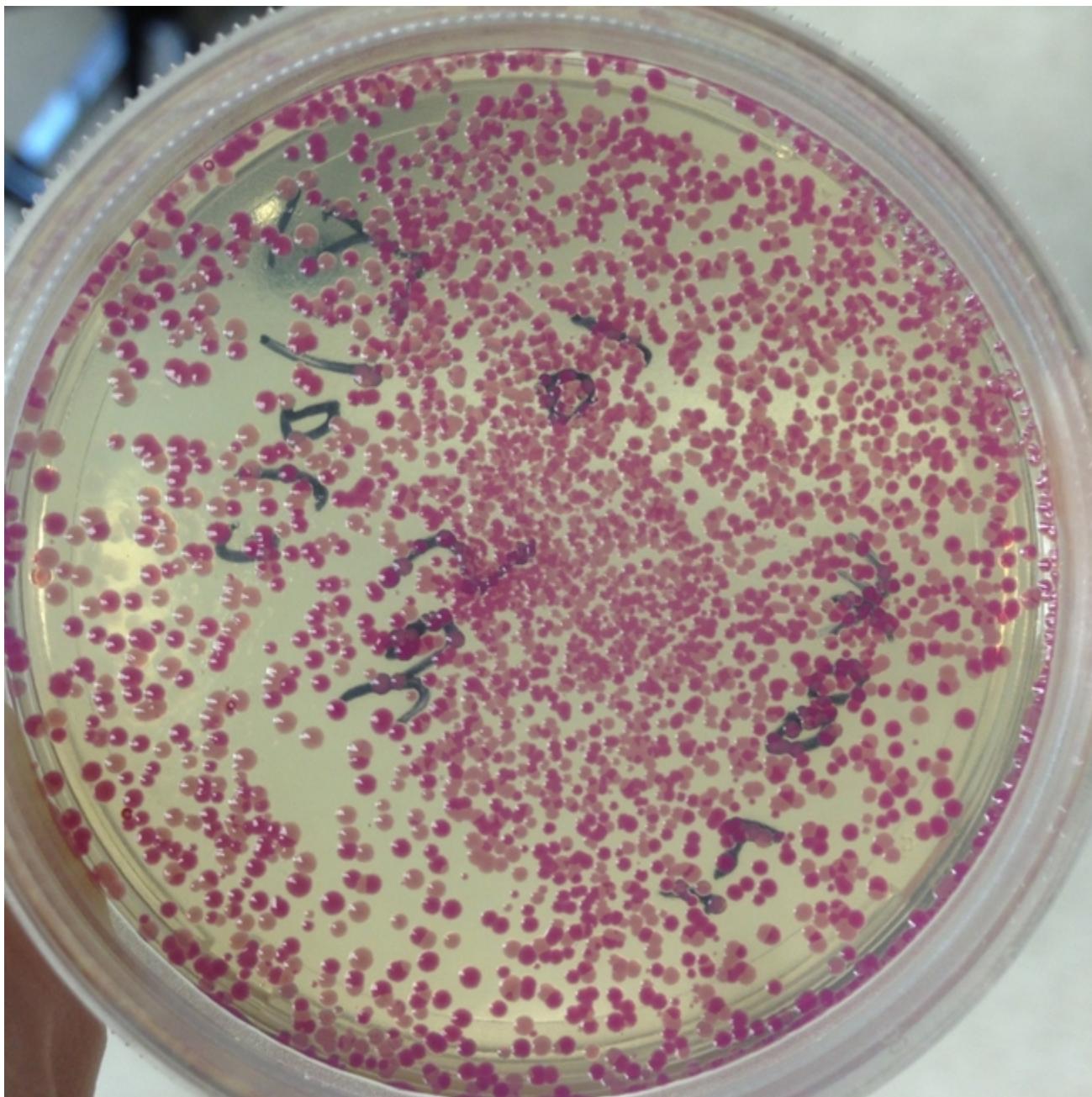
Near-lawns of colonies produced for all plates with >100 ng miniprep DNA as input. No colonies for iGEM kit plasmids.

Transformation efficiency of approximately 5×10^5 cfu/ug. Expression issue still unresolved at 24 hour incubation mark (all J04450 colonies equally intense bright red, most K314100+E1010 colonies bright red, some dim red, most K314100+RFPyy colonies white with some dim red).

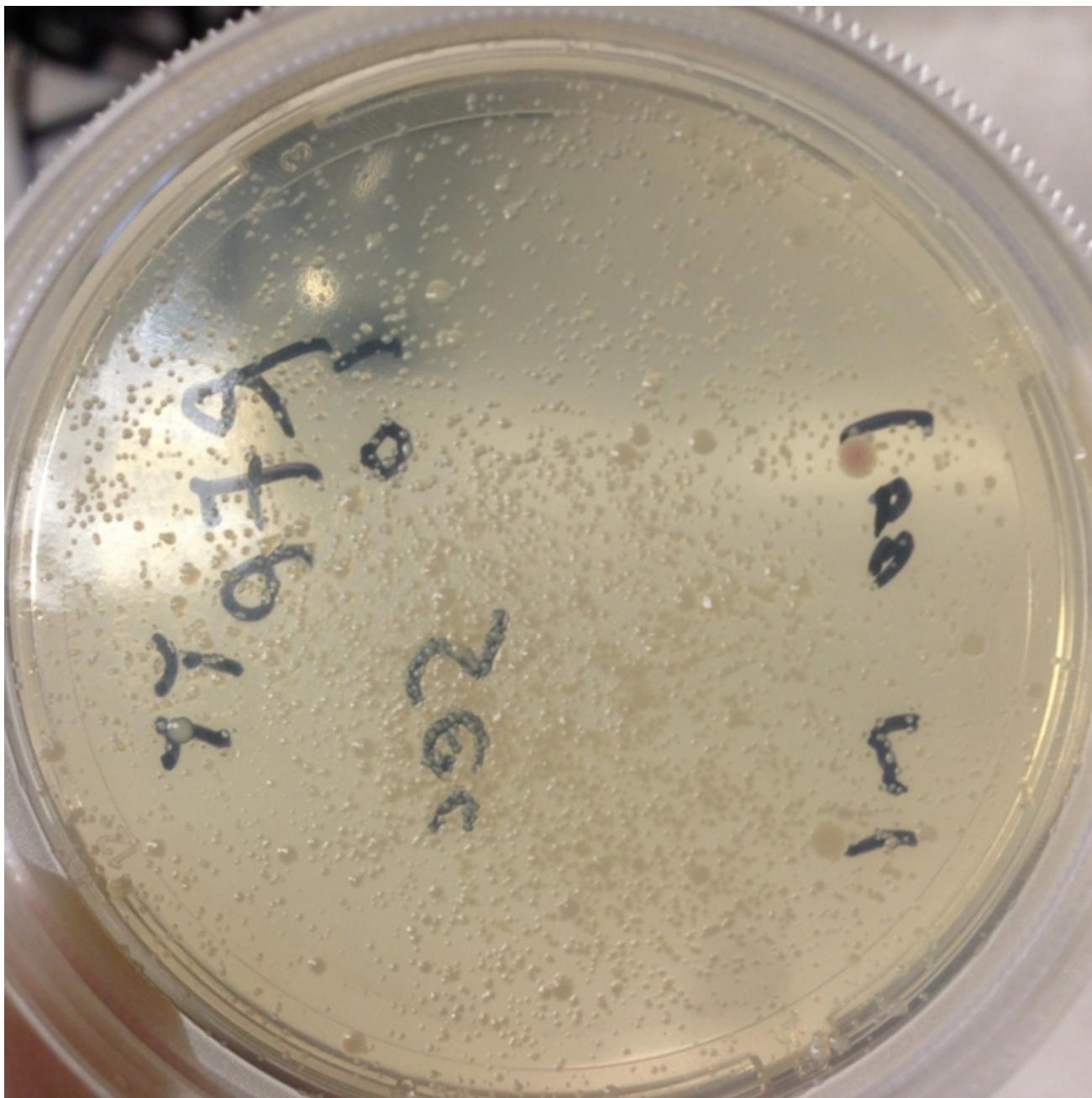
Control J04450 expression in BL21



Expression in K314100+E1010



Expression in K314100+RFPyy



Experiment Attached Images

J04450_(1024x1024).jpg

K314100_E1010_(1024x1024).jpg

K314100_RFPyy_(1024x1024).jpg

Experiment: 2015-06-28 Making Competent DH5 alpha Cells

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFPyy Expression Tests | Owner: Jarrod Shilts

Purpose: Treat cells with ions to induce chemical competence for future transformatiosn

Ingredients

- E. coli strain (DH5 α , BL21, TOP10, dam-/dcm-, etc.)
- LB Media
- 100 mM CaCl₂
- 100 mM MgCl₂
- 85 mM CaCl₂, 15% glycerol v/v
- ddH₂O
- Liquid Nitrogen

Experiment Procedures

Preparing Chemically Competent Cells

Steps

#	Title	Timer
1	Streak out a stock of cells on a LB plate and grow overnight at 37°C	16:00:00
2	Pick a single colony and make a 10 ml starter culture to grow overnight at 37°C	16:00:00
3	Inoculate 1 L of LB with the starter culture and grow at 37°C until an OD ₆₀₀ of 0.35-0.4, approximately 3-4 hours (measure OD every 30 min until 0.2, then every 15-20 minutes)	4:00:00
4	At the correct OD, remove the culture from the incubator and chill on ice for 20-30 minutes, swirling every 5 minutes for even cooling (Place 250 ml centrifuge bottles on ice to cool)	00:30:00
5	Split the culture into four parts by pouring 250 ml into chilled centrifuge bottles and pellet by centrifuging at 3000 g for 15 min at 4°C	00:15:00
6	Decant supernatant and resuspend each pellet in 100 ml of ice cold 100 mM MgCl ₂ (combine suspensions in two bottles)	00:00:00
7	Centrifuge at 2000 g for 15 min at 4°C	00:15:00
8	Decant supernatant, resuspend each pellet in 200 ml of ice cold 100 mM CaCl ₂ , and incubate on ice for 20 minutes (start cooling 100 microcentrifuge tubes at this time)	00:20:00
9	Centrifuge at 2000 g for 15 min at 4°C (during this spin rinse a 50 ml conical with ddH ₂ O and cool on ice)	00:00:00

10	Decant supernatant, resuspend each pellet in 50 ml of ice cold 85 mM CaCl ₂ , 15% glycerol v/v, and transfer to chilled 50 ml conical	00:00:00
11	Centrifuge at 1000 g for 15 min at 4°C	00:00:00
12	Decant supernatant and resuspend each pellet in 2 ml of ice cold 85 mM CaCl ₂ , 15% glycerol v/v (final OD ₆₀₀ should be 200-250)	00:00:00
13	Aliquot 50 µl of suspension into chilled microcentrifuge tubes and snap freeze in liquid nitrogen, then store at -80°C until needed	00:00:00

Note: Other media can be substituted for LB (SOB, 2xYT, etc.)

Note: Glassware should be detergent free as detergent reduces cell competency

Note: OD should not be any higher than 0.4. In case of a higher, redilute the culture and start over

Note: After growth stage, cells should always be kept at 4°C as well as any solutions and containers should be pre-chilled on ice

Tip: Use good sterile procedure when plating and growing liquid culture due to the absence of any antibiotic

Tip: Work in the cold room using ice buckets to ensure correct temperature, especially when aliquoting

Linked Resources

- E. coli Chemical Competence (Protocol)

Experiment: **2015-06-29 DH5 alpha transformations**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **RFPyy Expression Tests** | Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	From iGEM 2014 distribution, resuspend parts in 10 ul	00:00:00
	R0010 Lac repressible strong promoter	
	K863005 E coli laccase with T7 promoter	
	K525998 T7 promoter with RBS	
2	To competent cells made 6/28, add 2 ul of newly resuspended parts (400 pg), 1 ul of E1010 RFP miniprep (130 ng), 1 ul RFPyy gel lig 1 (130 ng), and 1.2 ul K11280 (240 pg)	00:00:00
3	Incubate cells on ice 30 minutes. Heat shock 50 seconds at 42 degrees.	00:00:00
4	Outgrowth in 350 ul SOC for 45 min. Plate 400 ul on large plates. All on CMR except K11280, which was plated 300 ul of Amp plate and 100 ul on small 1/2X CMR plate.	00:00:00

Experiment Results

Near lawn of colonies produced for E1010 and RFPyy. Between 2-50 colonies for other parts, except K863005, which failed to produce colonies on either the Amp or Cmr plates. Transformation efficiency of approximately 3×10^6

Experiment: 2015-07-01 - Promoter Miniprep

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFPyy Expression Tests | Owner: Daniel Shaykevich

Purpose: Isolate whole plasmid from E. coli cells

Ingredients

- E. coli liquid culture
- Viogene Mini Plasmid DNA Extraction System
- ddH₂O @ 65°C

Experiment Procedures

Plasmid DNA Extraction (Viogene)

Steps

#	Title	Timer
1	Take 5 ml of overnight E. coli culture and pellet by centrifuging at 3,000 rpm for 10 min (transfer to 15 ml conical if not already in one)	00:00:00
2	Decant media and resuspend in 200 µl MX1 Buffer by vortexing or pipetting	00:00:00
3	Transfer to 1.5 ml microcentrifuge tube	00:00:00
4	Add 250 µl MX2 Buffer and invert 4-6 times to mix	00:00:00
5	Incubate at room temperature for 3 min (do not allow to lyse for more than 5 min)	00:04:00
6	Add 350 µl MX3 Buffer to neutralize lysate and invert 4-6 times to mix	00:00:00
7	Centrifuge at 13,000 rpm for 10 min	00:10:00
8	Transfer supernatant into a Mini column that is in a collection tube and let stand for at least 1 minute	00:00:00
9	Centrifuge at 9,000 rpm for 60 sec and discard flow-through	00:01:00
10	Wash the column with 500 µl WN Buffer by centrifuging at 9,000 rpm for 60 sec and discard flow-through	00:01:00
11	Wash the column with 700 µl WS Buffer by centrifuging at 9,000 rpm for 60 sec and discard flow-through	00:01:00

12	Centrifuge column at 13,000 rpm for 3 min to remove residual ethanol	00:03:00
13	Place the column in a microcentrifuge tube and add 50 µl warm ddH ₂ O to the center of the membrane	00:00:00
14	Incubate at room temperature for 5 min	00:05:00
15	Centrifuge at 13,000 rpm for 5 min to elute DNA	00:03:00
16	Properly label and confirm DNA presence by Nanodrop and place in 4°C refrigerator	00:00:00

Tip: If there is an expectation of low yield or to increase yield, run the flow-through from step 9 through the column again before discarding

Experiment Results

Miniprep of 5 ml cultures of the following parts: E1010A, E1010B, K525998 (x3), K863005 (x2), RFP YY (x2), R0010A, R0010B, R0010C

Linked Resources

- Miniprep (Viogene) (Protocol)

Experiment: 2015-07-02 Adding R0010 promoter to RFPyy

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFPyy Expression Tests | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Digest 4 ul of J04500 miniprep with SpeI and PstI in NEBuffer 2.1. Digest 5 ul of RFPyy (7/1 miniprep) with XbaI and PstI in NEBuffer 2.1	00:00:00
2	Run digest on gel and gel extract with Qiagen kit. Elute in 25 ul. Nanodrop.	00:00:00
3	Add 10.2 ul of RFPyy (70 ng), 6.8 ul of J04500 (80 ng) to 20ul T4 ligase reaction with 1 ul ligase. Incubate overnight at 16 degrees	00:00:00
4	Transform DH5 alpha with 5 ul of ligation product. Two transformation per ligation mixture. Also transform 3 ul of BioBrick parts K516032, K540001, B0034, B0032, and B0015	00:00:00
5	Viogene Miniprep- K516032, J04500 1A and J04500 1B, B0032, B0034. Yield all above 100 ng/ml	00:00:00

Experiment Results

>20 colonies formed on all plates. No RFP visible without induction

Milestone: Spectrophotometer OD/RFP Calibration

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Type here the description for this folder, what experiments are to be done and what figures are expected

Experiment: 2015-06-12 Setting up dilutions

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Spectrophotometer OD/RFP Calibration | Owner: Jarrod Shilts

Experiment Procedures

First procedure

Steps

#	Title						Timer
1	From 3 ml liquid culture of J04450 grown for 24 hours, aliquot into 96 well plate						00:00:00
	J04450	200 ul	175 ul	150 ul	125 ul	100 ul	
	LB Diluent	0 ul	25 ul	50 ul	75 ul	100 ul	
2	Take 600 nm absorbance and RFP fluorescence measurements on CDB core third floor plate reader spectrophotometer						00:00:00
3	Dilute 1 ul in 999 ul LB for each sample, then dilute 1 ul of that dilution in 999 ul of LB. Plate 100 ul as 1:10^6 dilution. Dilute 10 ul of 1:10^6 dilution in 90 ul LB. Plate 100 ul as 1:10^7 dilution. Spread with spreaders, let media sink for 15 minutes, then incubate						00:00:00

Experiment: **2015-06-12 Counting colonies**

**Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Spectrophotometer
OD/RFP Calibration | Owner: Jarrod Shilts**

Experiment Procedures

First procedure

Steps

Experiment Results

No linear relationship found. Replication needed

Milestone: T4 PDG Assay for RFPyy

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: 2015-07-02 T4 PDG with UV of 100 and 1000 mJ/cm2

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: T4 PDG Assay for RFPyy | Owner: Jarrod Shilts

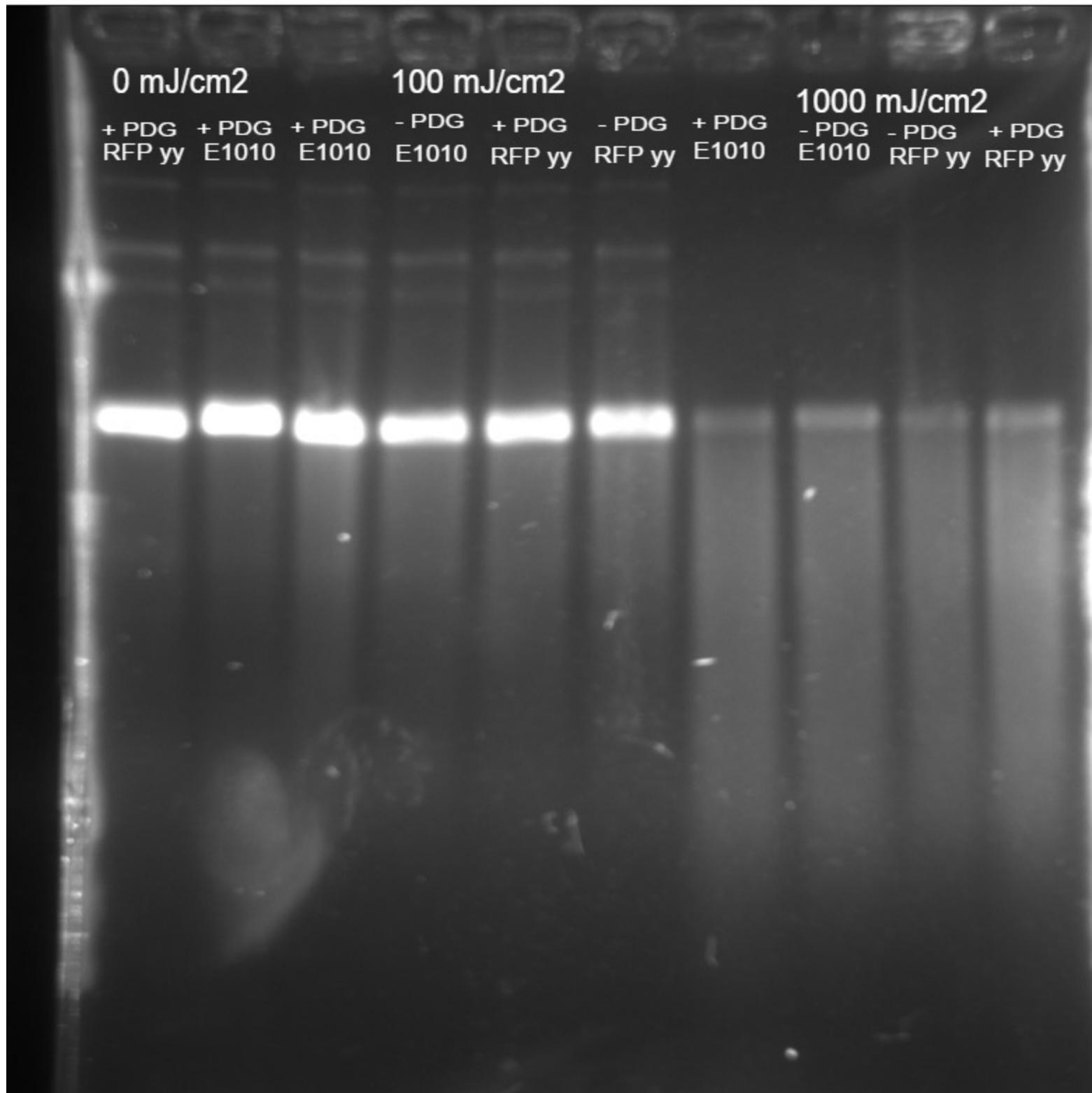
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Place 200 ng of PCR purified RFPyy or E1010 in 5 ul total volume of water. UV irradiate at 0, 100, or 1000 mJ/cm ² in crosslinker box.	00:00:00
2	Make mix of 118 ul water, 20 ul 10x PDG buffer, and 2 ul 10x BSA. Add 14 ul of mix to each tube of DNA. Then add either 1 ul of T4 PDG enzyme (+ condition) or 1 ul of water (- condition)	00:00:00
3	Place at 37 degrees for 20 hours.	00:00:00
4	Dissolve 0.6 g of agarose in 46 ml water. After cooling to 52 degrees, add 5 ml of 10x alkaline gel buffer. Pour at 4 degrees. After 15 min, submerge in 1x alkaline gel buffer.	00:00:00
5	Add 4.5 ul of alkaline loading buffer to samples. Heat samples to 60 degrees for 5 min, then bring to 4 degrees. Load on gel	00:00:00
6	Run at 50 V for 200 minutes at 4 degrees. Submerge in stopping solution for 45 minutes, then place in 50 ml 1x TAE with 5 ul Diamond nucleic acid dye for 20 minutes.	00:00:00

Experiment Results



Experiment Attached Images

7-3-15_pdg_rfp.jpg

Experiment: **2015-07-04 T4 PDG at 50 and 500 mJ/cm2**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **T4 PDG Assay for RFPyy** | Owner: **Jarrod Shilts**

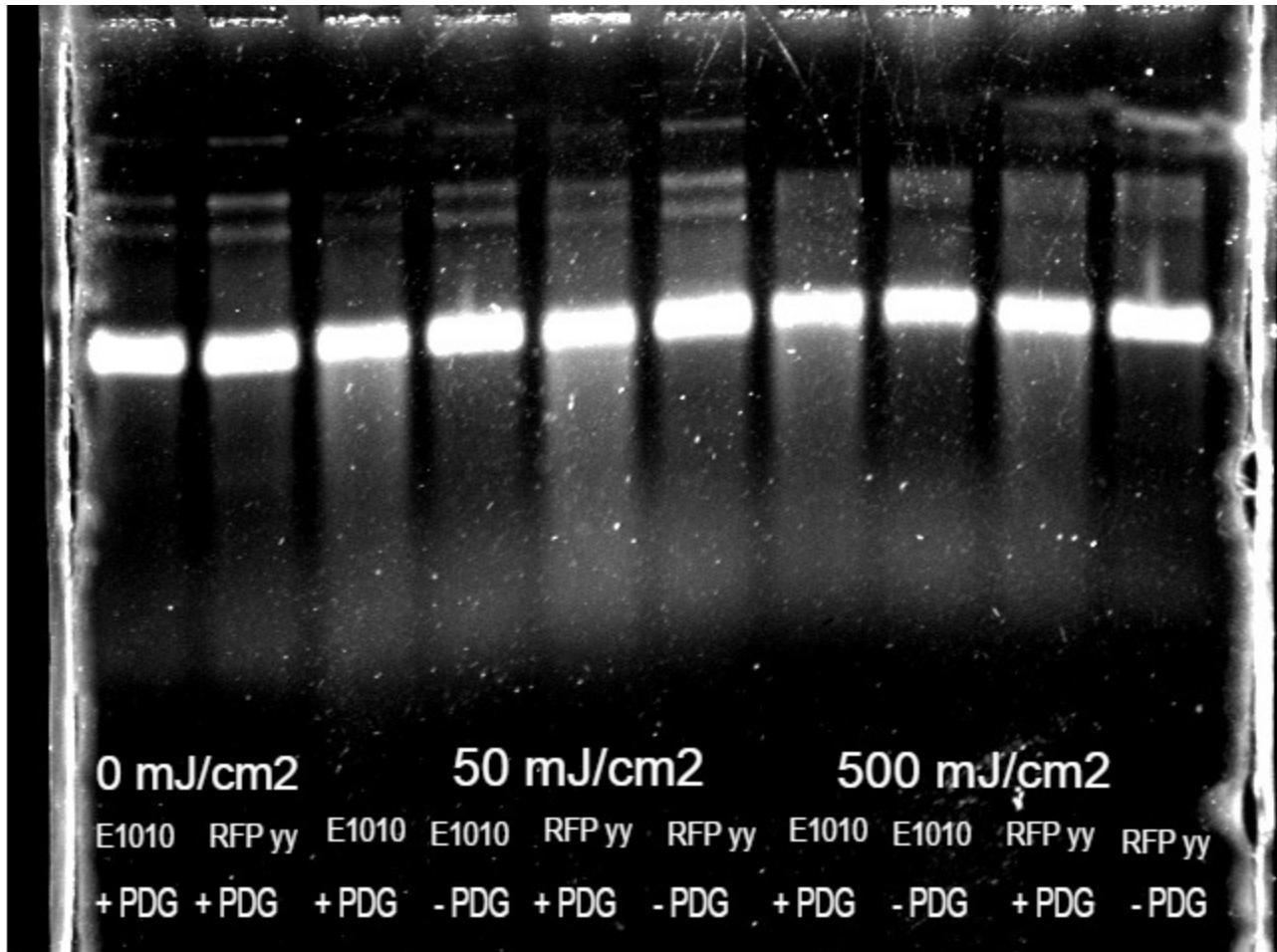
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 200 ng of PCR purified RFP gene + K314100 promoter to microcentrifuge tube. Adjust volume to 10 ul with water.	00:00:00
2	Expose to either 0, 50, or 500 mJ/cm ² of UV. To tube, add 9 ul of mix made of 20 ul 10x PDG buffer, 2 ul 100x BSA, and 68 ul water. Add either 1.3 ul of T4 PDG or 1.3 ul of water.	00:00:00
3	Incubate on thermocycler at 37 degrees for 12 hours, then place at 4 degrees. Freeze at -20 for temporary storage.	00:00:00
4	Disolve 0.52 g of agarose in 47 ml of water. Carefully bring to boil in microwave. Stir and cool until 50 degrees, then add 5.1 ml of 10x alkaline loading buffer. Cast gel at 4 degrees.	00:00:00
5	Add 28 ml of 10x alkaline loading buffer to 274 ml of DI water. Cool to 4 degrees then submerge solidified gel. Incubate at 4 degrees for 1.5 hours	00:00:00
6	Add 6 ul of 6x alkaline gel loading buffer. Mix at room temperature for 5 min. Load all of each sample on gel.	00:00:00
7	Run at 60 V at 4 degrees for 130 minutes. When done, remove gel, rinse briefly in 1x TAE, then incubate in stopping solution for 30 minutes at room temp.	00:00:00
8	Replace solution with 40 ml of 1x TAE with 4.3 ul of SYBR Safe added. Shake at room temperature for 15 minutes, image, then an additional 40 minutes, and image again.	00:00:00
9	Quantify smear on TIFF file with ImageJ	00:00:00

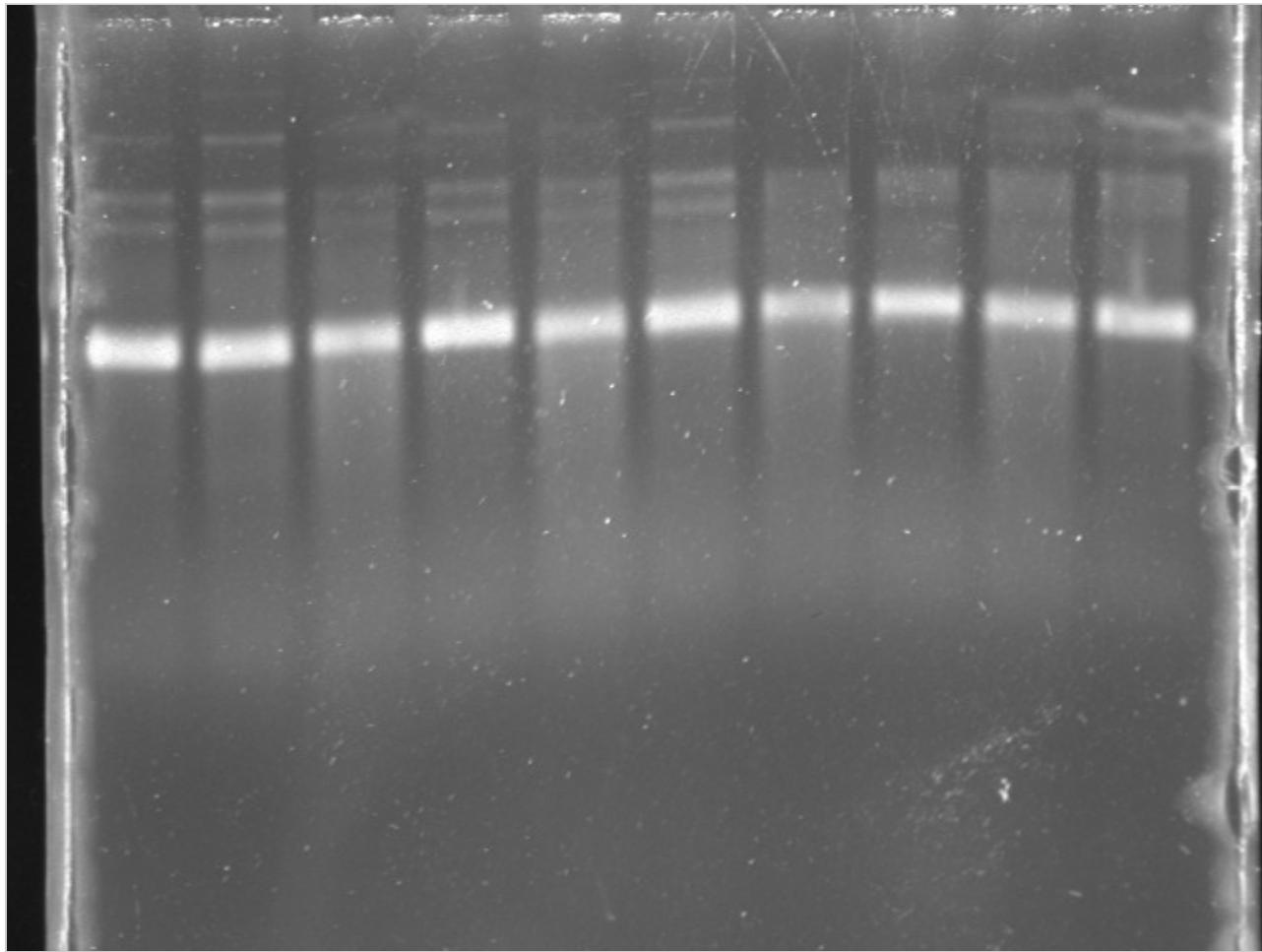
Experiment Results



Experiment Conclusion

RFPyy quantification slightly greater than E1010 for 50 mJ/cm², and slightly less for 500 mJ/cm². Higher UV dose does not produce higher values

Experiment Attached Images



15-07-04_pdg_with_rfp.tif

15-07-04_pdg_with_rfp.jpg

Experiment: 2015-07-06 PDG Assay, 4 replicates

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: T4 PDG Assay for RFPyy | Owner: Jarrod Shilts

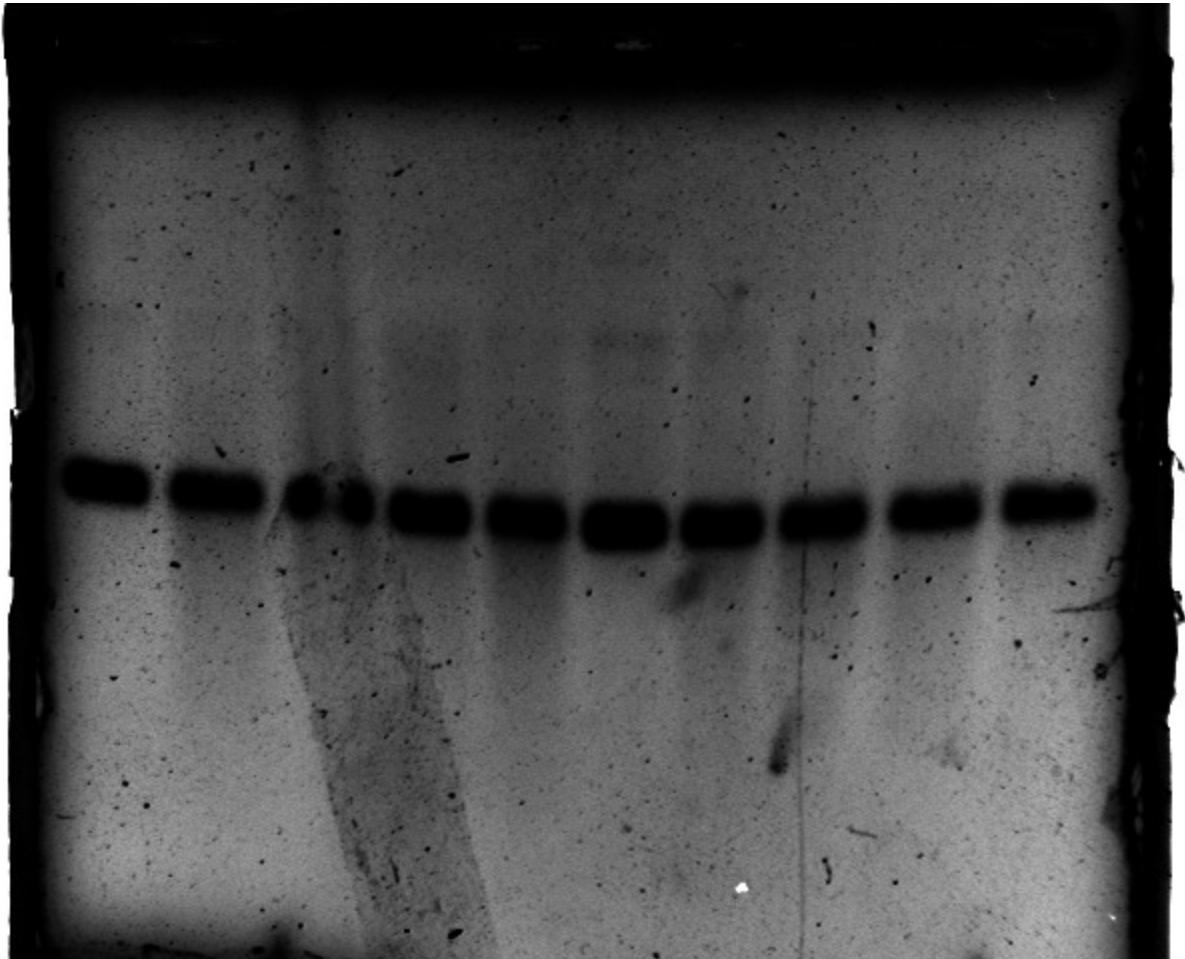
Experiment Procedures

New Procedure

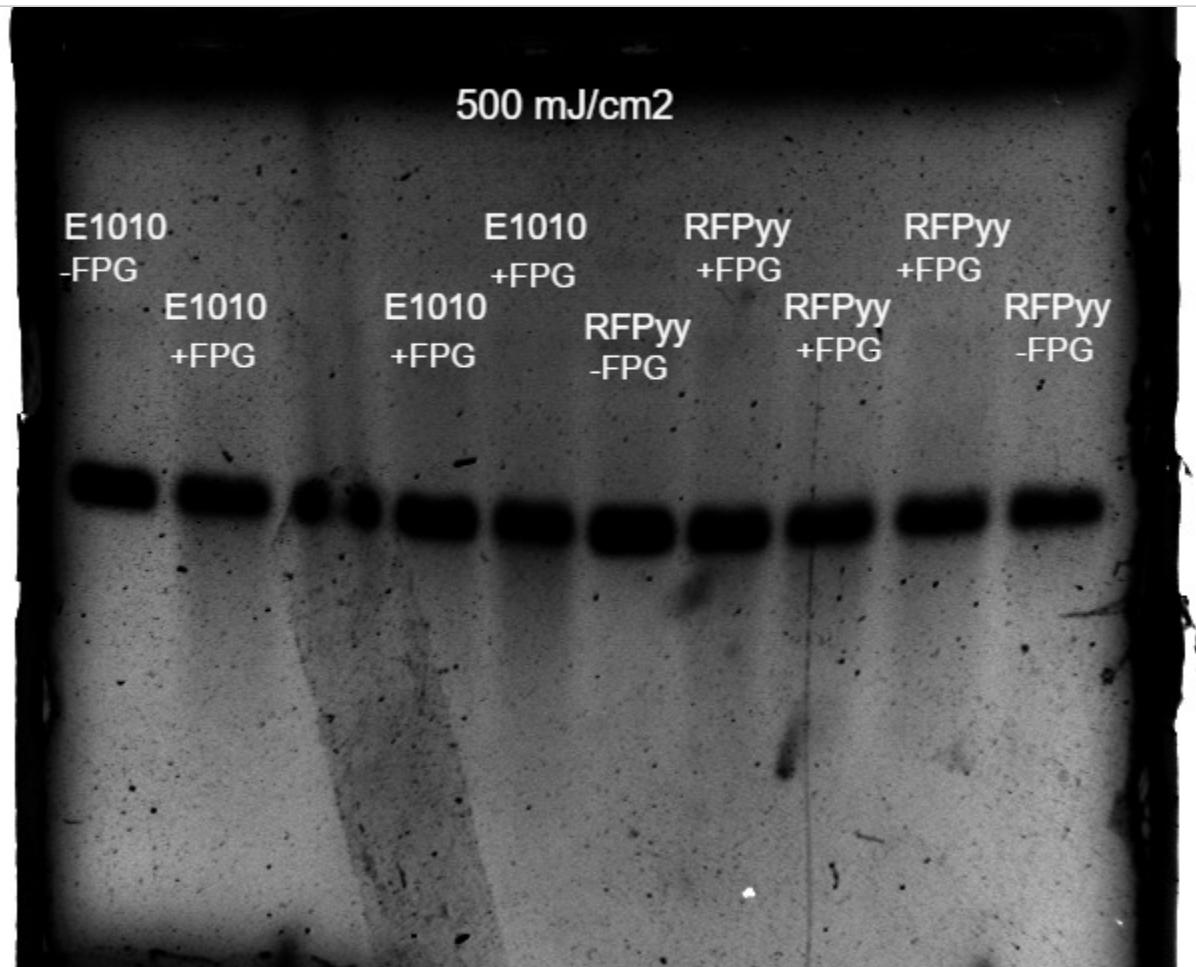
Steps

#	Title	Timer
1	Add 100 ng of PCR purified K314100+E1010 or K314100+RFPyy to tube with total volume of 5 ul of water. Irradiate at 0 or 5000 J/m2 UV	00:00:00
2	Set up mix for 10 ul total volume PDG reaction. Add 0,5 ul of T4 PDG or water.	00:00:00
3	Incubate at 37 degrees for 10 hours	00:00:00
4	Cast 1% alkaline gel with 10x buffer. Submerge in alkaline solution overnight at 4 degrees.	00:00:00
5	Add 4.5 ul of 6x alkaline loading buffer. Leave at room temp for 10 min. Load all 15 ul onto gel. Run at 60 V for 160 min at 4 degrees.	00:00:00

Experiment Results



Experiment Attached Images



15-07-06_pdg.tif

Milestone: Optimized Part Collection

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

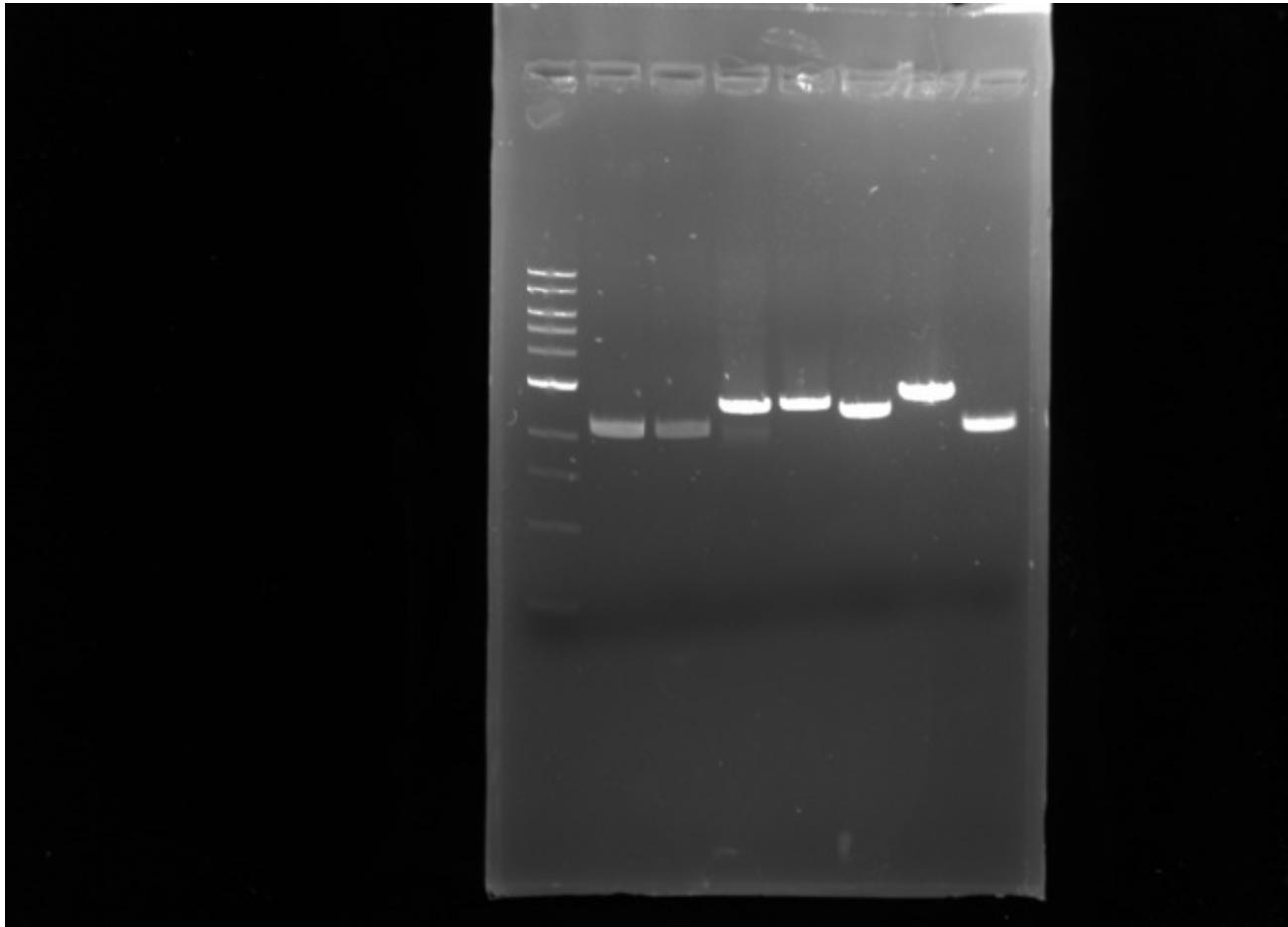
Experiment: 2015-09-14 gBlock ligation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Optimized Part Collection | Owner: Jarrod Shilts

Experiment Procedures

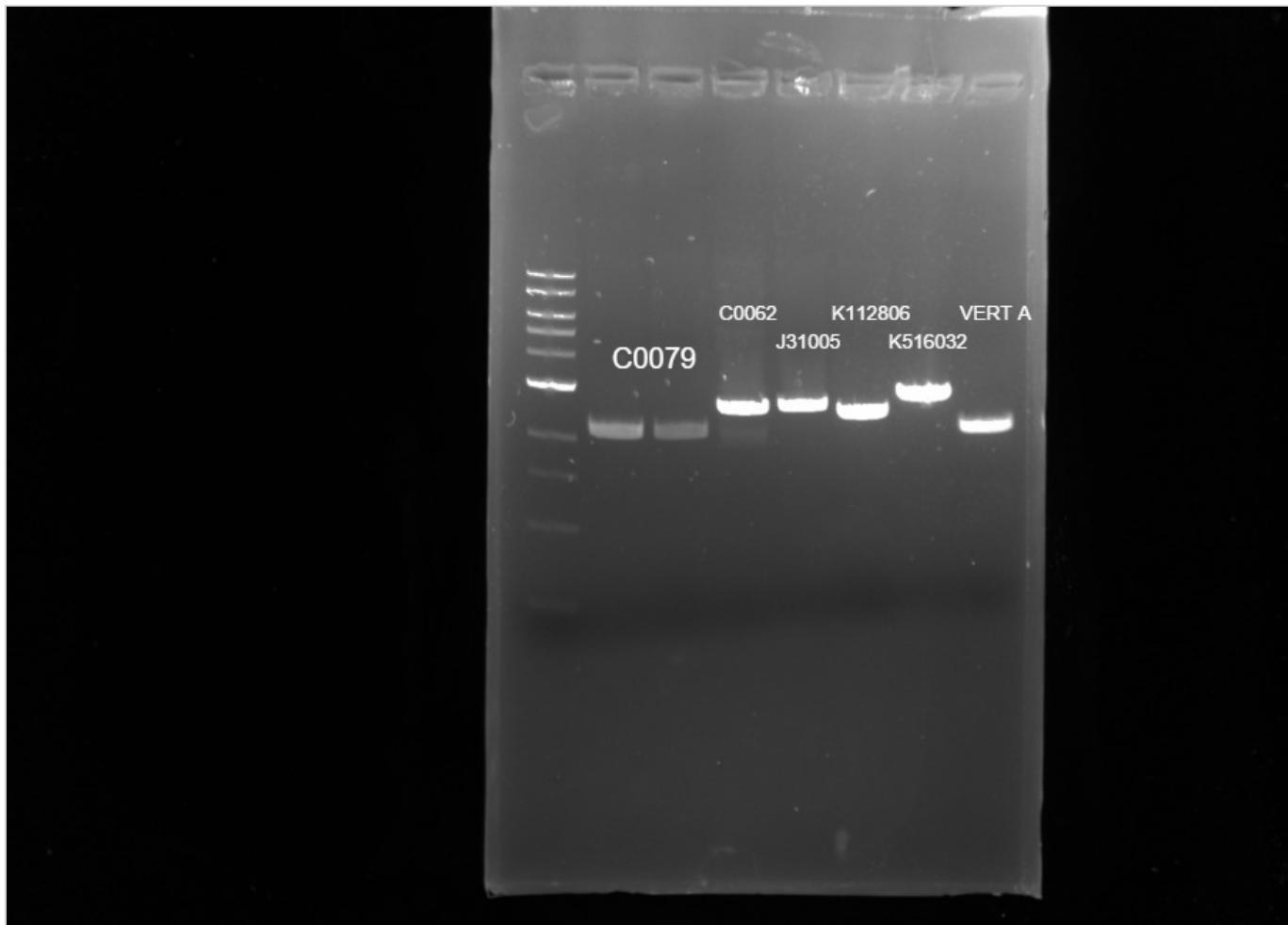
New Procedure

Experiment Results



Incorrect on VERT A, and both C0079. Rest correct. K516032 not part of collection

Experiment Attached Images



15-09-16_part_collection_conf1.tif

Milestone: **T7 Ura3 Homology Assembly**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: **2015-08-24 T7 and Ura3 PCR**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: T7 Ura3 Homology Assembly | Owner: Jarrod Shilts

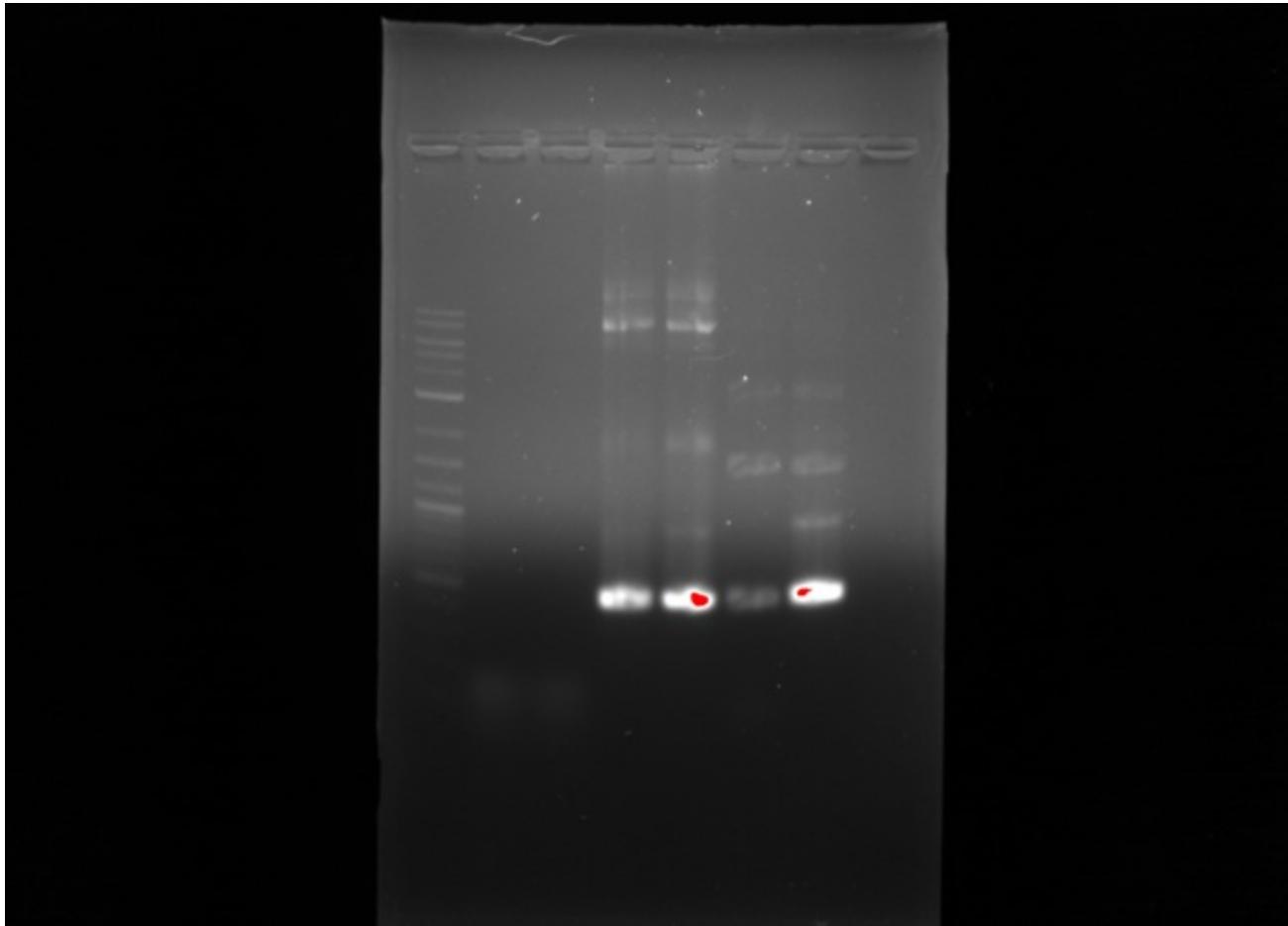
Experiment Procedures

New Procedure

Steps

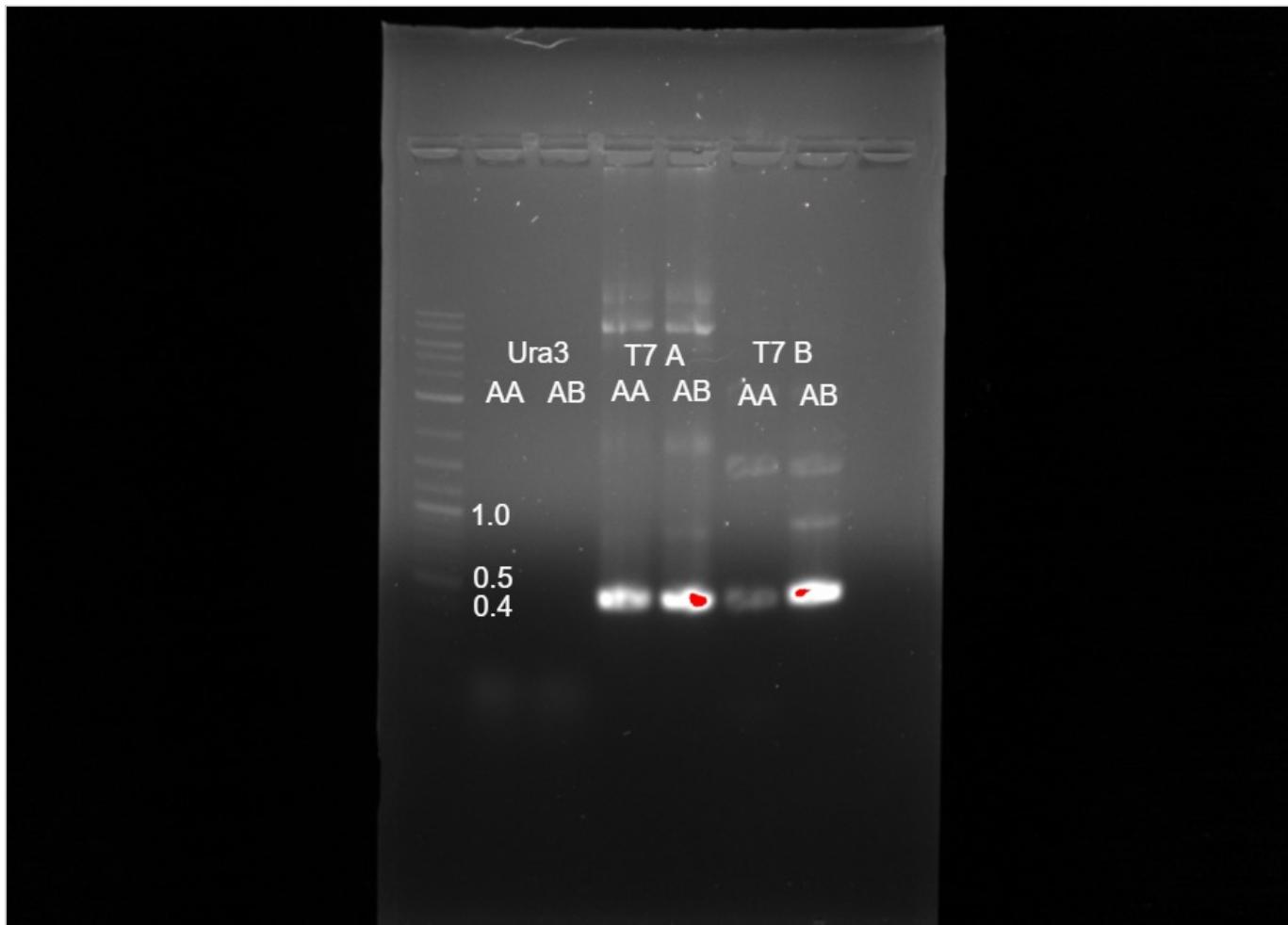
#	Title			Timer
1	50 ul platinum taq PCR reaction with 1 ul of template DNA			00:00:00
	FWD primer	REV primer	Template	
1	FWD_Ura3_with_T7A_HR	REV_Ura3_with_T7A_HR	200 alpha	
2	FWD_Ura3_with_T7A_HR	REV_Ura3_with_T7B_HR	200 alpha	
3	FWD_T7A_HRgal1_extension	REV_T7A_HRgal1_with_ura3	T7 A (B)	
4	FWD_T7A_HR_with_Ura3	REV_T7A_HRgal1_extension	T7 A (B)	
5	FWD_T7A_HRgal1_extension	REV_T7A_HRgal1_with_ura3	T7 B (A)	
6	FWD_T7B_with_ura3	REV_T7B_HRgal1_extension	T7 B (A)	
2				00:00:00
3				00:00:00

Experiment Results



1 and 2 fail to produce product. Others produce correct size (~500bp) along with extra bands

Experiment Attached Images



15-08-24_gibson_ura3_pcr.tif

Experiment: 2015-08-25 Extractions

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: T7 Ura3 Homology Assembly | Owner: Jarrod Shilts

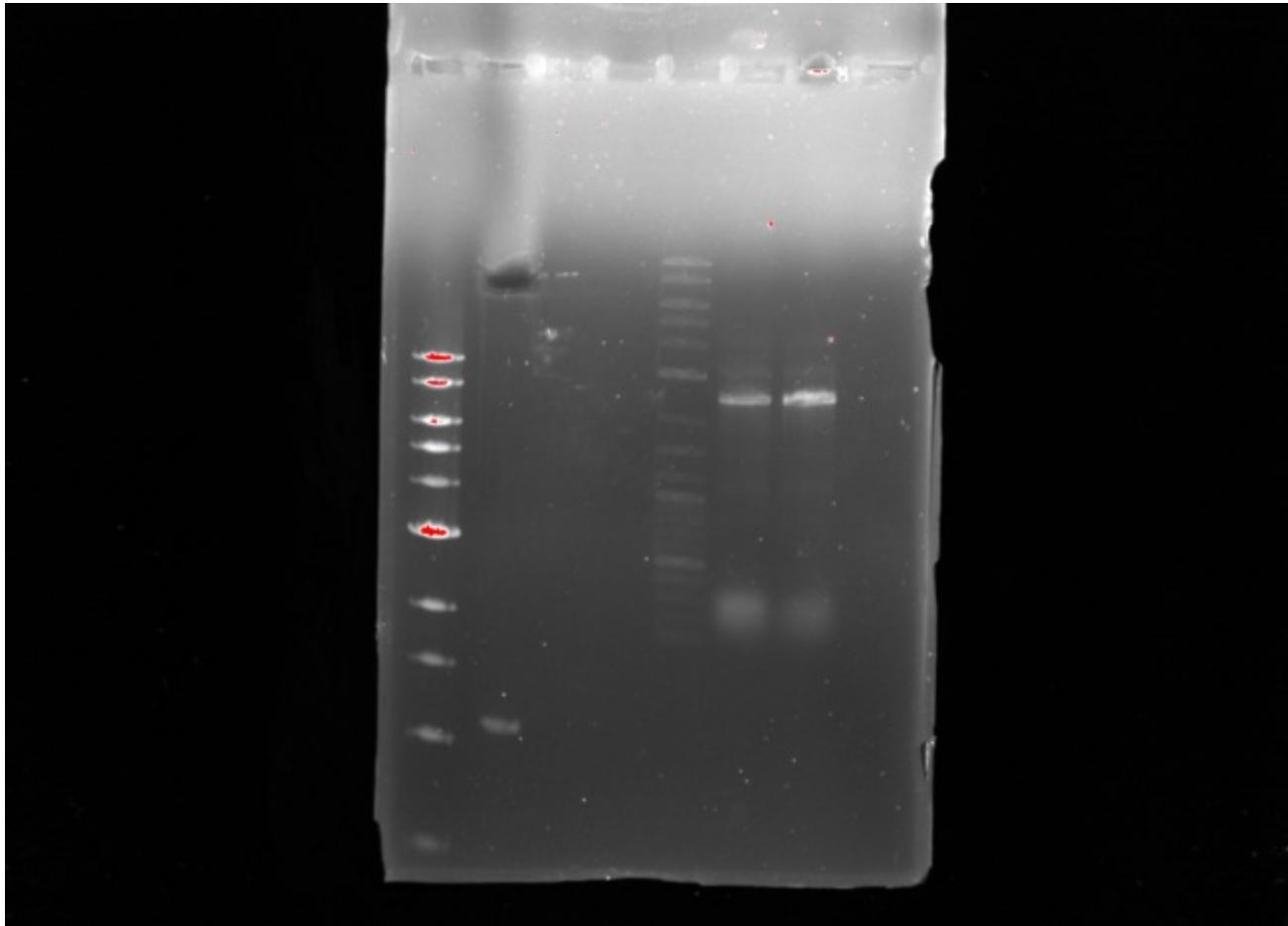
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Gel extract correct bands from 8/24 with Qiagen kit. Elute in 20 ul	00:00:00
2	Re-do Ura3 PCR with Ta=52, 35 cycles platinum taq 50 ul reaction. Use alpha 199 as template DNA	00:00:00
3	Gel extract Ura3 bands (2.7 kb) from PCR product	00:00:00

Experiment Results



Correct Ura3 bands (on right) with some faint extra bands

Experiment Attached Images



15-08-25_ura3_gib_pcr_check.tif

Milestone: VERT Assembly

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: 2015-09-04 Vector extraction

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: VERT Assembly | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Digest 12 ul of R0010 with XbaI and SpeI in cutsmart. Incubate 37 min 2 hours	00:00:00
2	Run all of sample on 1% gel. Extract band at 2070 bp with Qiagen kit. Elute 30 ul	00:00:00
3		00:00:00

Experiment: **2015-09-07 Gibson assembly confirmation**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: VERT Assembly | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Experiment Results

Milestone: **Part Expression**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Owner: **Jarrod Shilts**

Experiment: **2015-08-22 RFP T7 ligation**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Part Expression** | Owner: **Jarrod Shilts**

Experiment Procedures

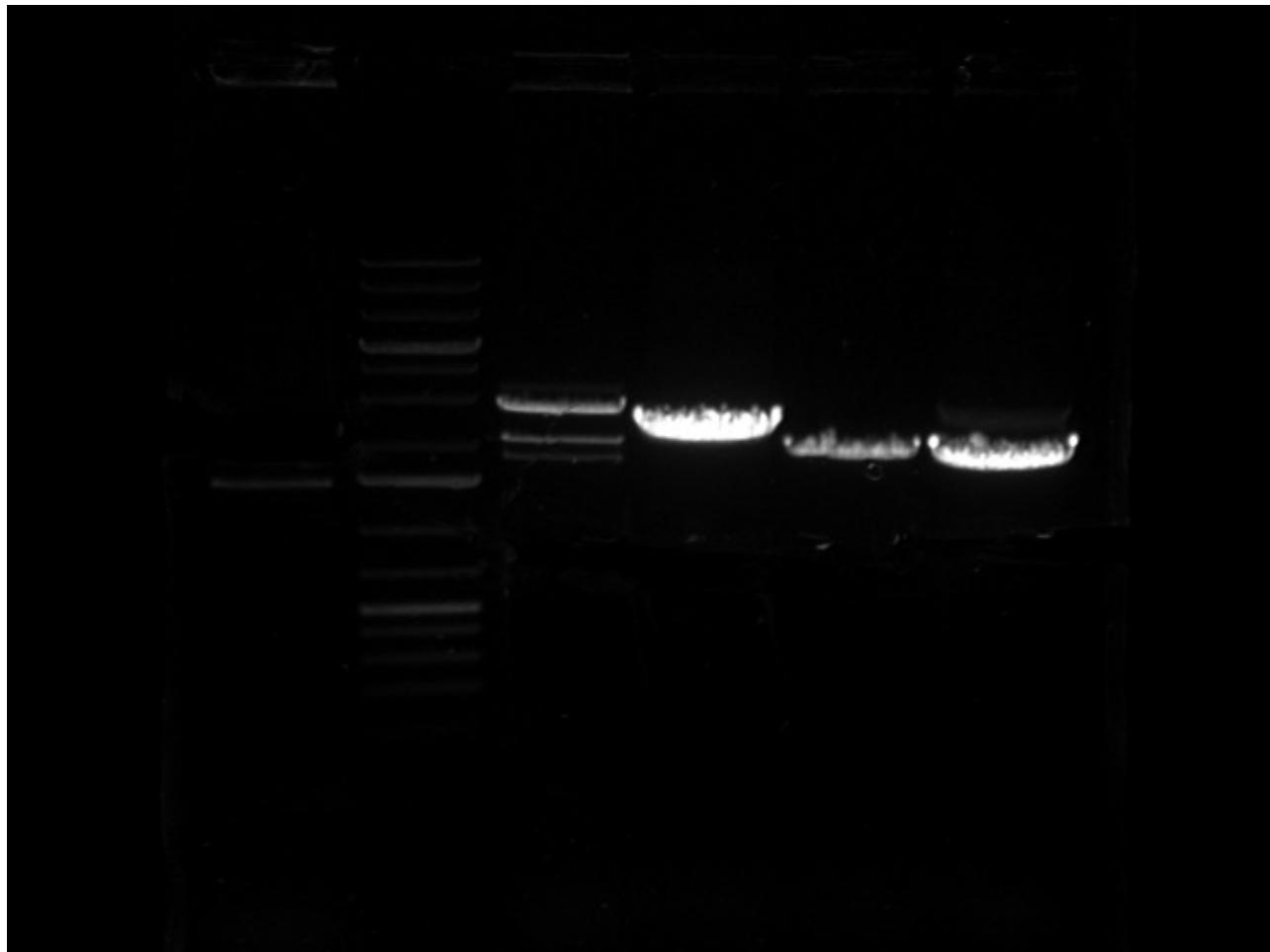
New Procedure

Steps

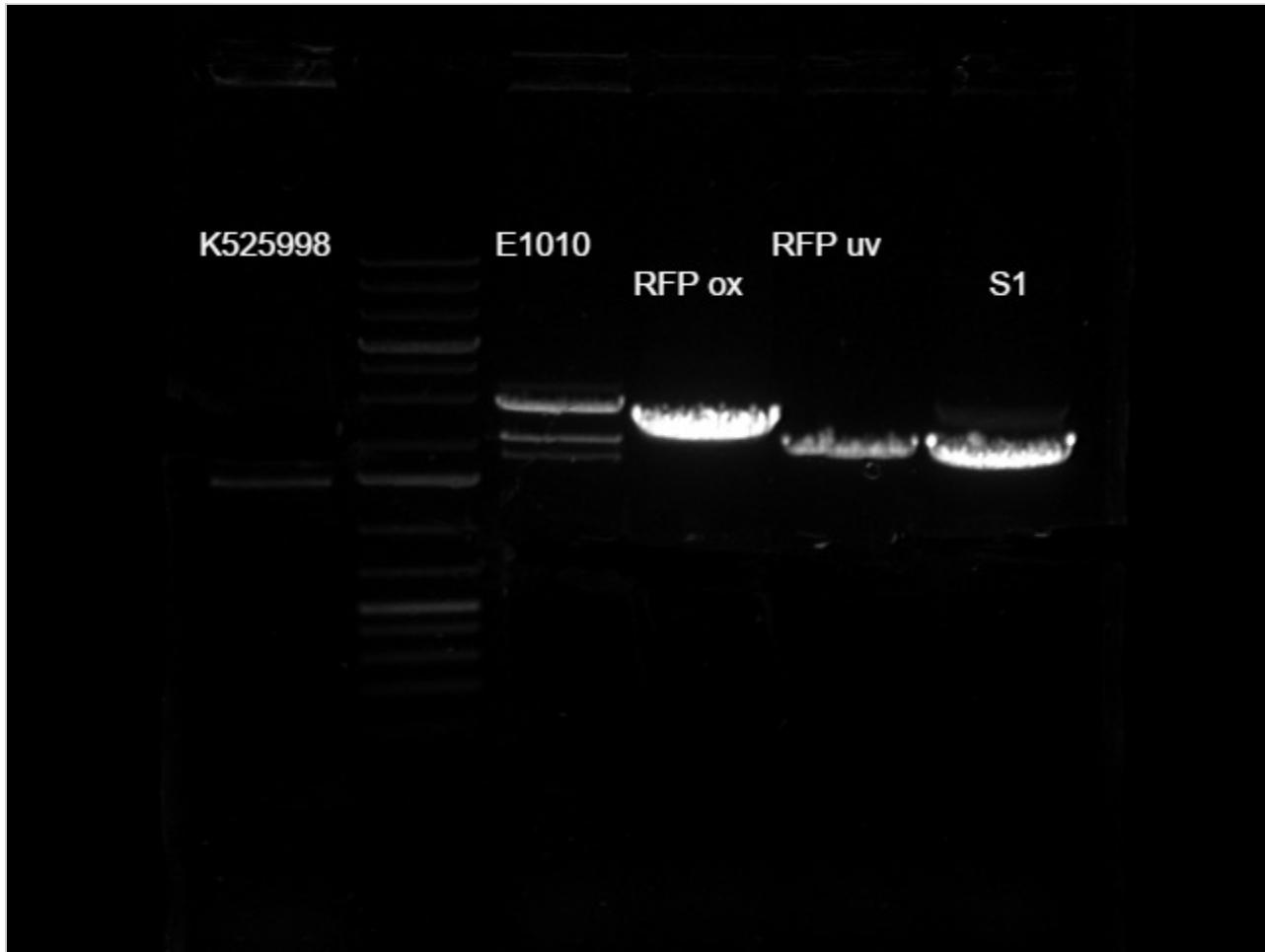
#	Title	Timer
1	Digest 6 ul of plasmid (~2 ug) in 20 ul reaction. Digest K525998 with PstI and SpeI, and RFP ox, RFP uv, E1010, and S1 Nuc with XbaI and PstI. Incubate 2 hours 37 degrees, then 20 min 80 degrees	00:00:00
2	Run all of digest on gel and gel extract using Qiagen kit. Elute K525 in 30 ul, others in 20 ul. All bands found except RFP ox (extracted instead region where should be)	00:00:00
3	Ligate with T4 DNA ligase. 14 ul insert + 3.2 ul vector for RFP ox and E1010 (~80 ng insert 100 ng vector), 10 ul RFP uv + 4 ul vector (200 ng insert), 5 ul S1 + 5 ul vector (250 ng insert). Ligate 16 hours 16 degrees	00:00:00
4	Transform into (DE3) BL21 cells	00:00:00

Experiment Results

No band visible for RFP ox (excised part of gel where should be). All others produce band around correct size



Experiment Attached Images



15-08-22_gel_extraction.tif

Experiment: 2015-08-26 T7 RFP expression

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Part Expression | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Make 3 ml liquid culture of each ligation and grow for 24 hours	00:00:00
2	Add 40 ul of 100 mM IPTG to culture and incubate 12 hours	00:00:00
3		00:00:00

Experiment Results

No RFP visible in any cultures, including E1010 with T7 promoter, neither before nor after IPTG induction

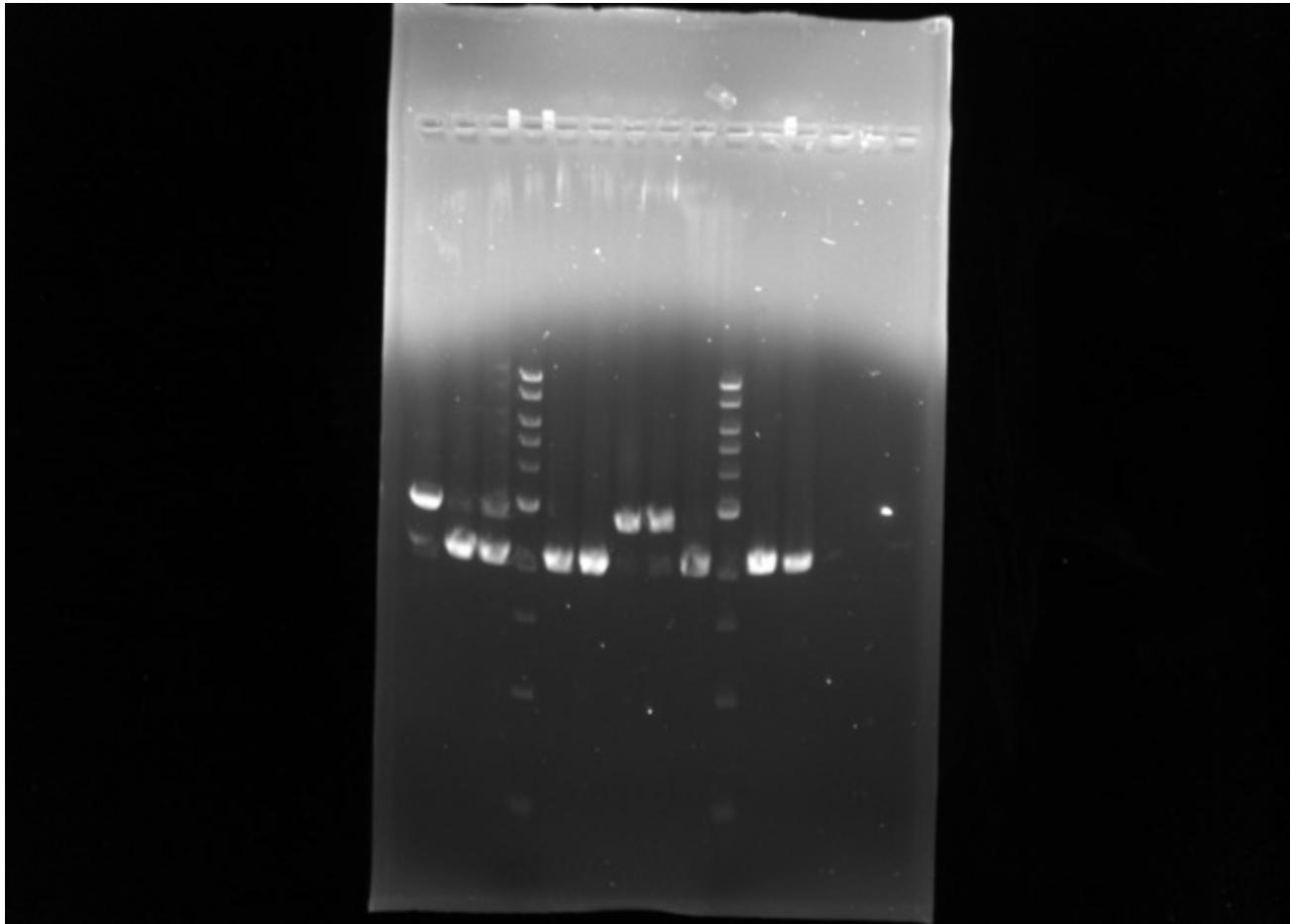
Experiment: 2015-08-28 T7 Ligation Check

Project: Modulating Evolutionary Potential with Rationally Designed Genes | **Milestone:** Part Expression |
Owner: Jarrod Shilts

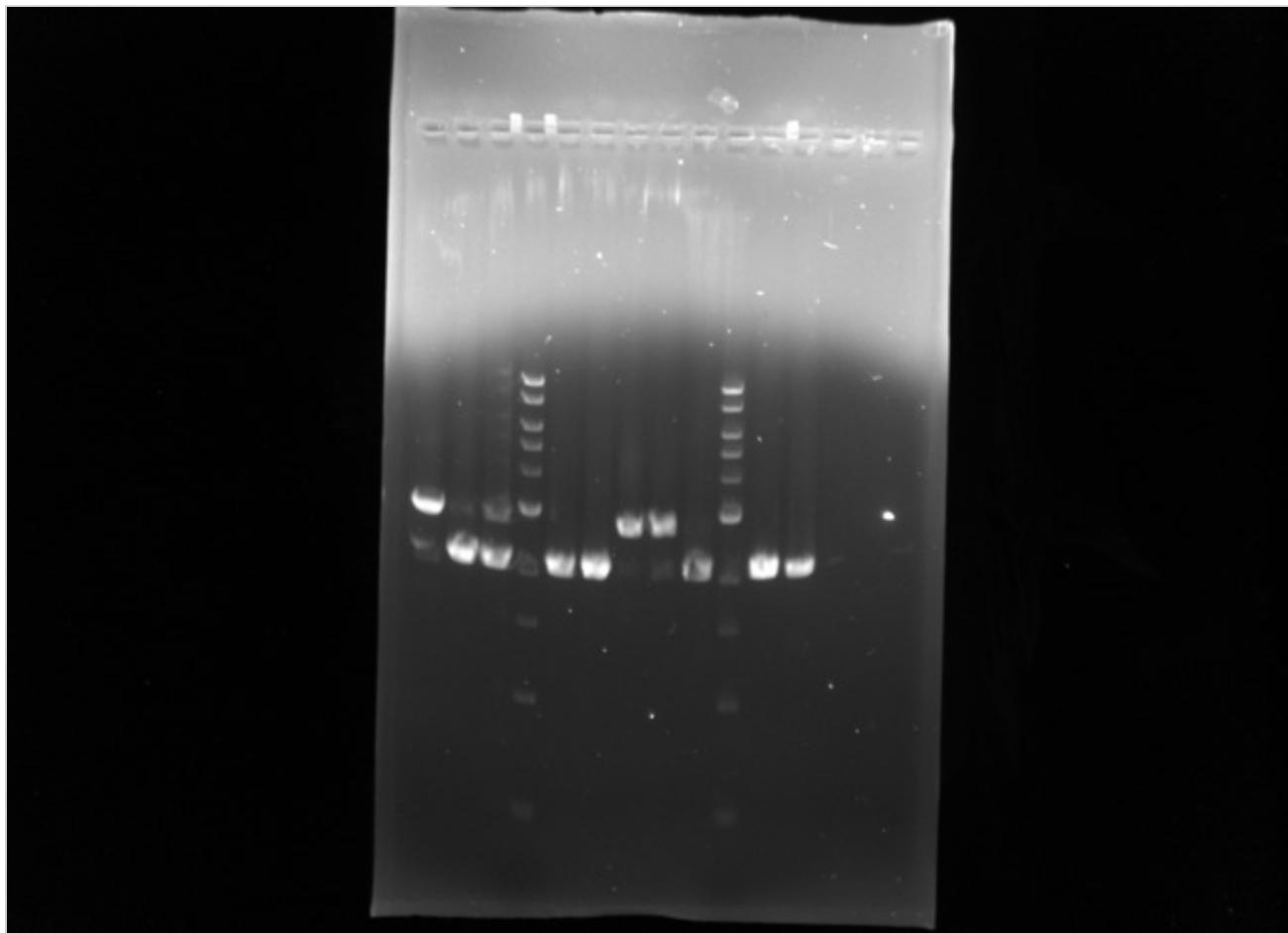
Experiment Procedures

New Procedure

Experiment Results



Experiment Attached Images



15-08-28_k25_ligation_conf.tif

Experiment: 2015-09-06 J04500 promoter assembly

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Part Expression | Owner: Jarrod Shilts

Experiment Procedures

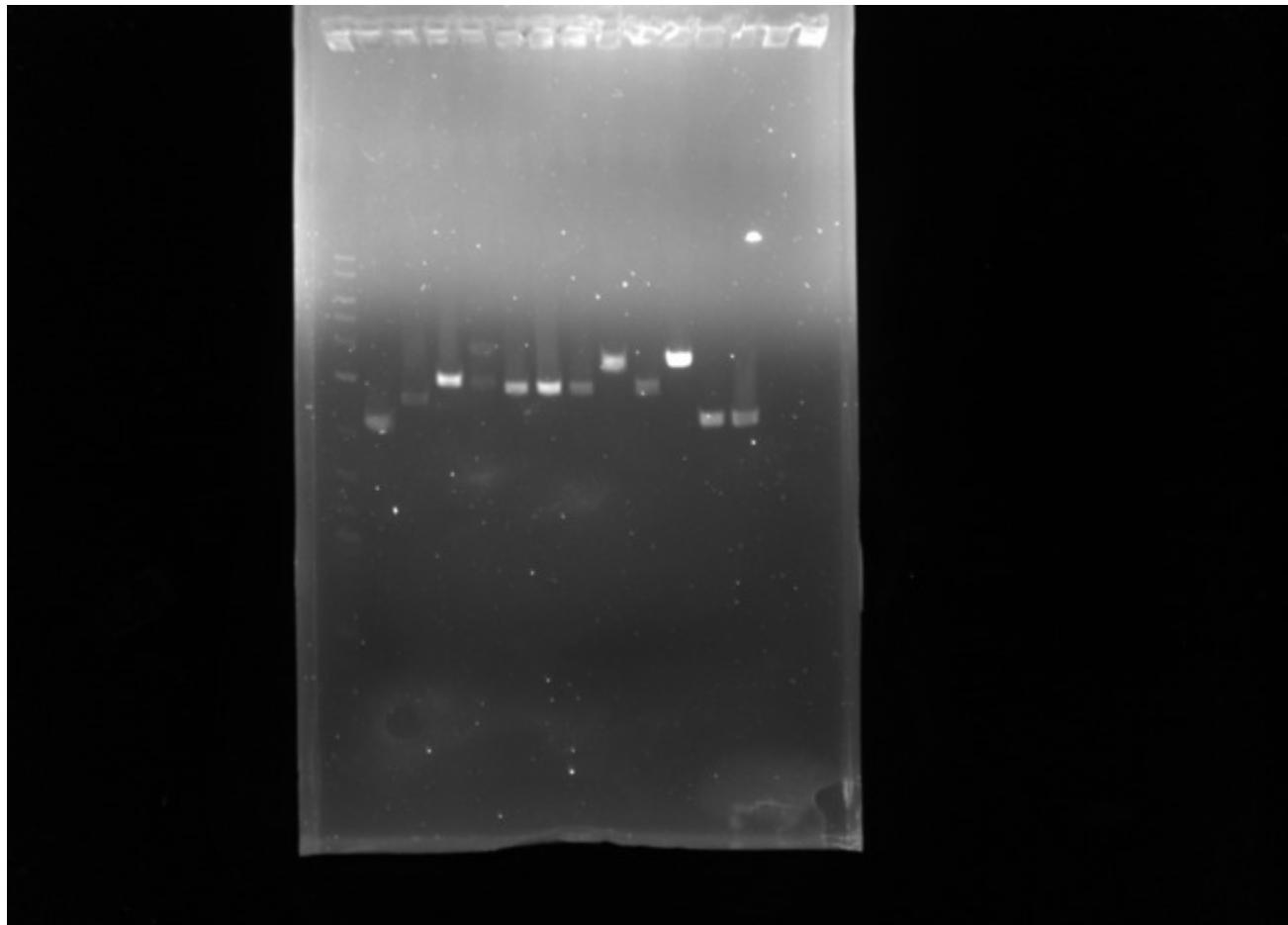
New Procedure

Steps

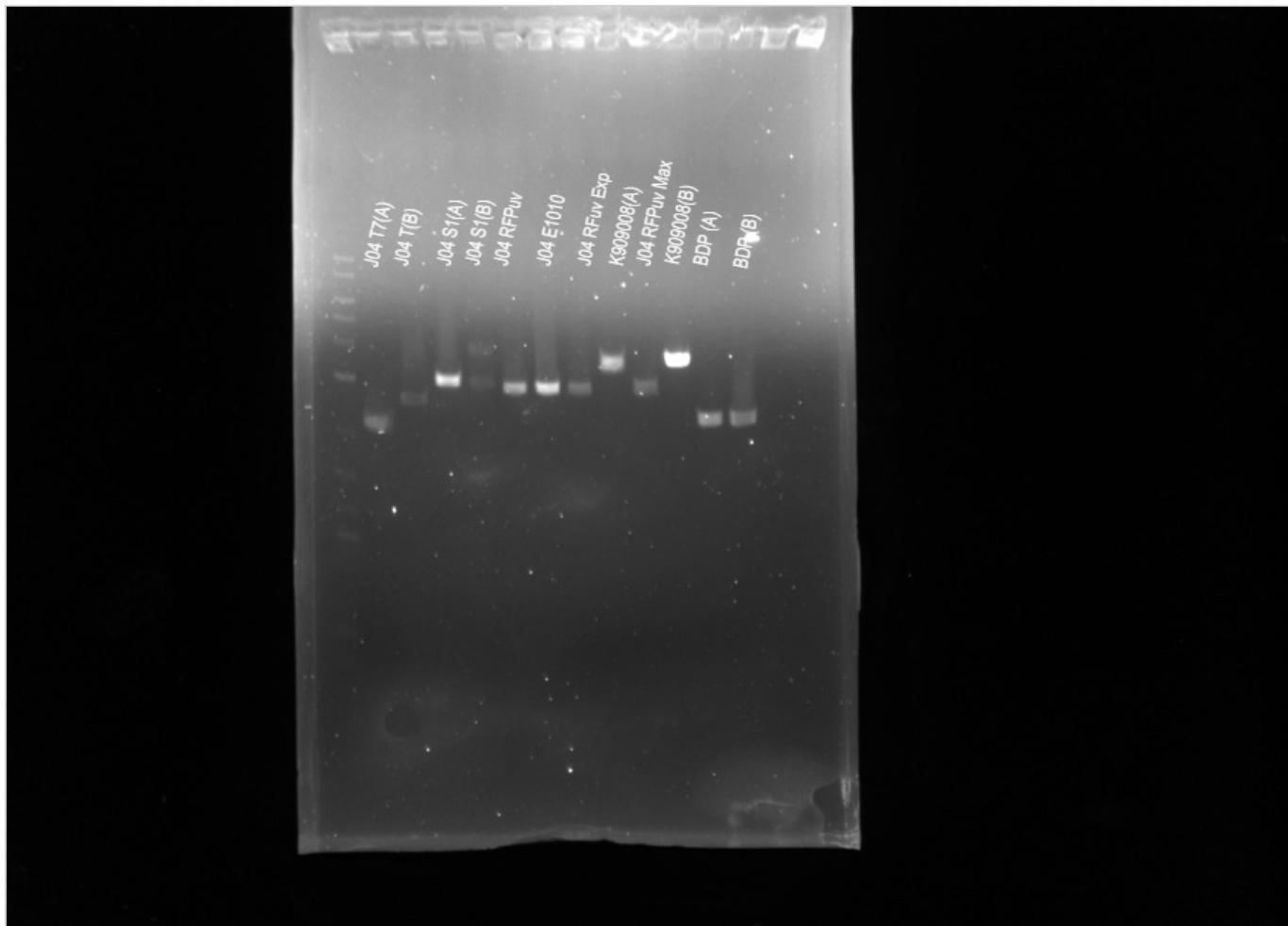
#	Title	Timer
1	BioBrick assembly	00:00:00
2		00:00:00
3		00:00:00

Experiment Results

Only incorrect are J04500 T7 (A) and J04500 S1 (B)



Experiment Attached Images



15-09-06_diagnostic.tif

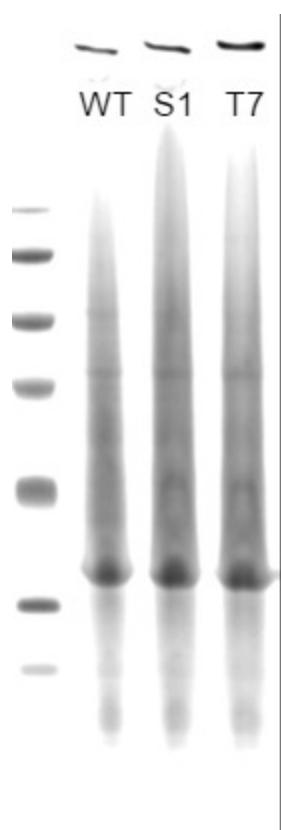
Experiment: **2015-09-15 S1 T7 Coomassie**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Part Expression** |
Owner: **Jarrod Shilts**

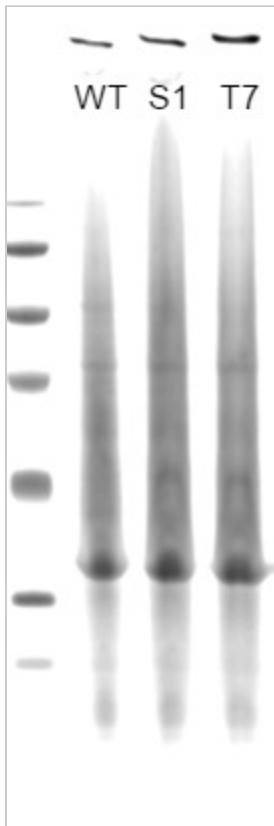
Experiment Procedures

New Procedure

Experiment Results



Experiment Attached Images



coomassie.png

Milestone: **RFP uv Experimentation**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Owner: **Jarrod Shilts**

Experiment: **2015-08-03 RFP uv PCR**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **RFP uv Experimentation** | Owner: **Jarrod Shilts**

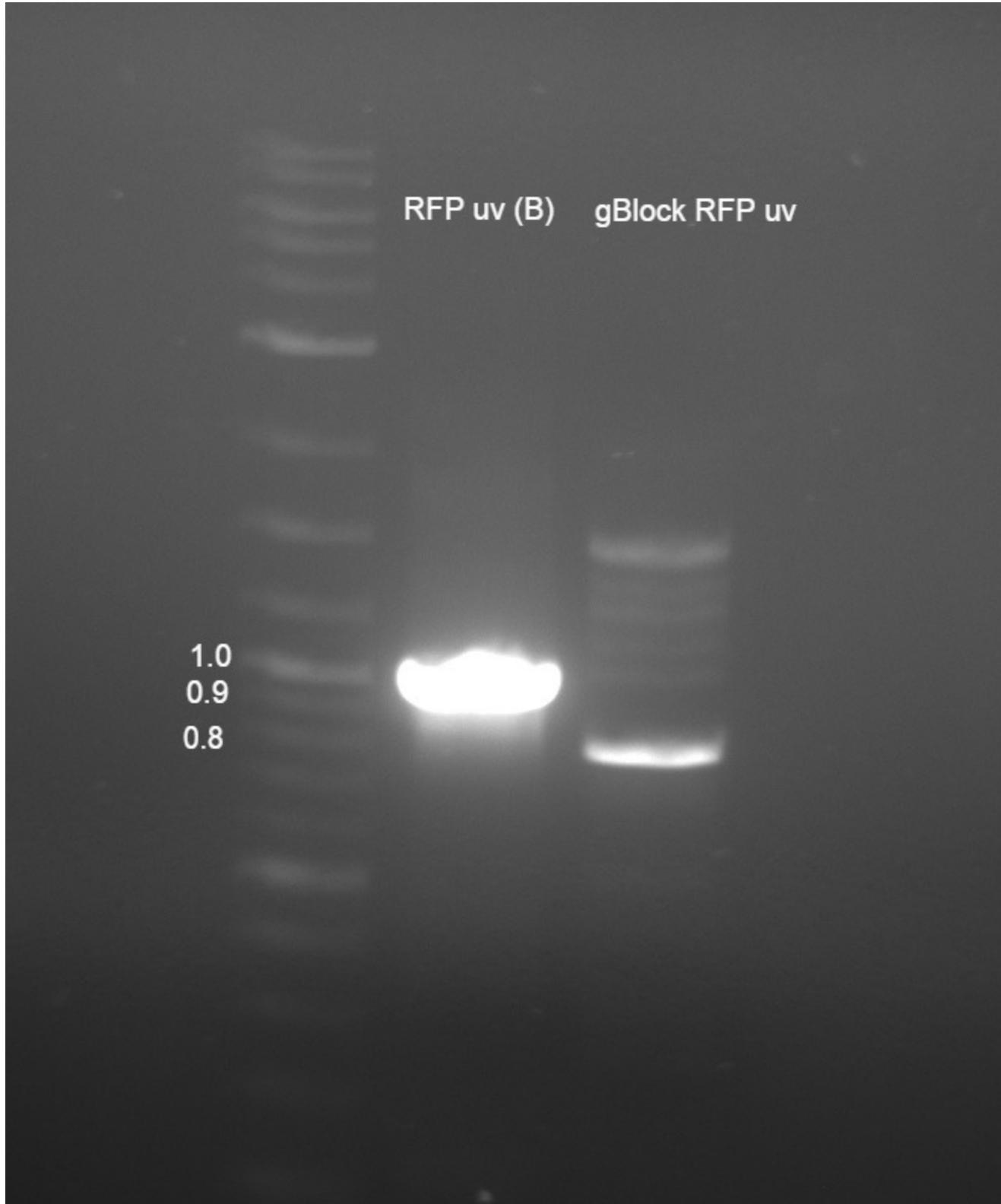
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Amplify 0.5 ul of miniprepped RFP UV (B) or 0.1 RFP uv gBlock in 25 ul Platinum taq PCR mix. Annealing temp 55 degrees, 30 cycles	00:00:00
2	Load 5 ul on to 1.2 % gel	00:00:00
3		00:00:00

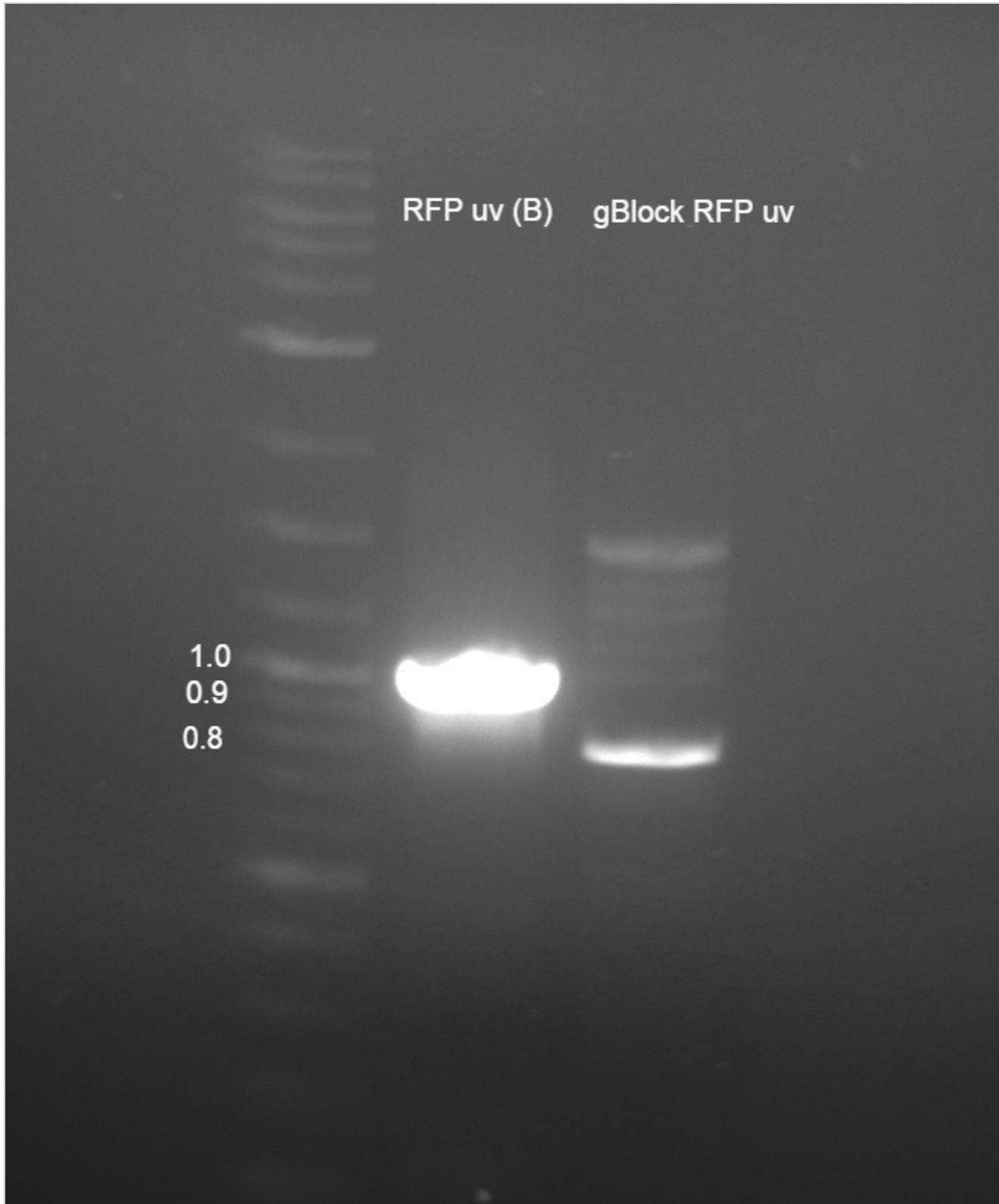
Experiment Results



Experiment Conclusion

RFP uv integrated into pSB1C3 successfuly and PCR extractedd

Experiment Attached Images



150802_rfp_uv_pcr.jpg

Experiment: **2015-08-18 RFP uv Extraction**

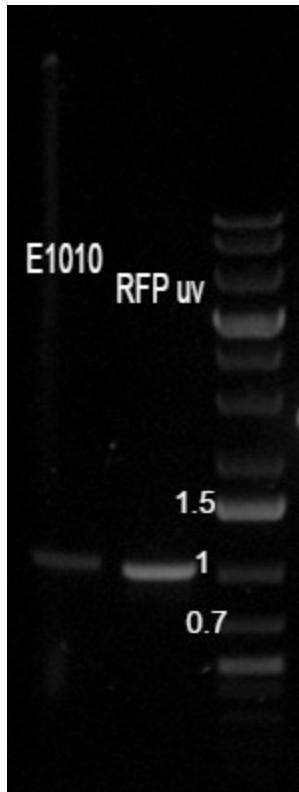
Experiment Procedures

New Procedure

Steps

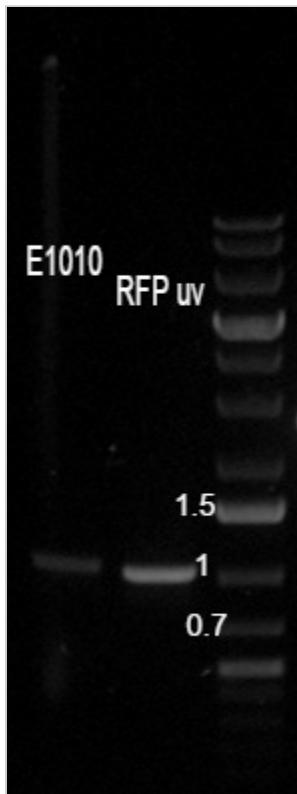
#	Title	Timer
1	Platinum Taq PCR with VF2 and VR primers on 25 ng of confirmed plasmid template. Ta=55 degrees, 35 cycle PCR reaction. Two 50 ul reactions per RFP	00:00:00
2	Combine two RFP reactions. Run 4 ul on 0.8% gel	00:00:00
3	PCR purify using Qiagen kit. Elute in 30 ul.	00:00:00

Experiment Results



Both RFP at correct size

Experiment Attached Images



15-08-19_RFP.png

Experiment: 2015-08-21 RFP uv alkaline gel

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFP uv Experimentation | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 200 ng of PCR product to total volume of 16.8 of water. Add 2 ul of T4 PDG reaction buffer. Irradiate all samples at 1000 uJ x 100 on Lee lab UV crosslinker	00:00:00
2	Add 0.2 ul 100x BSA, 1 ul PDG. Incubate 37 degrees overnight	00:00:00
3	Add 5 ul of 6x alkaline gel loading buffer. Heat samples 50 degrees for 5 min.	00:00:00
4	Cast 1.2% alkaline gel with 10x stock solution. Cool and incubate in alkaline gel solution 2 hours at 4 degrees	00:00:00
5	Load all 25 ul of sample and run gel 60 volts for 3 hours at 4 degrees	00:00:00
6	Rinse with TAE, then submerge in alkaline gel stopping solution 45 min.	00:00:00
7	Stain in TAE with 1x SyBBUREsafe dye and image	00:00:00

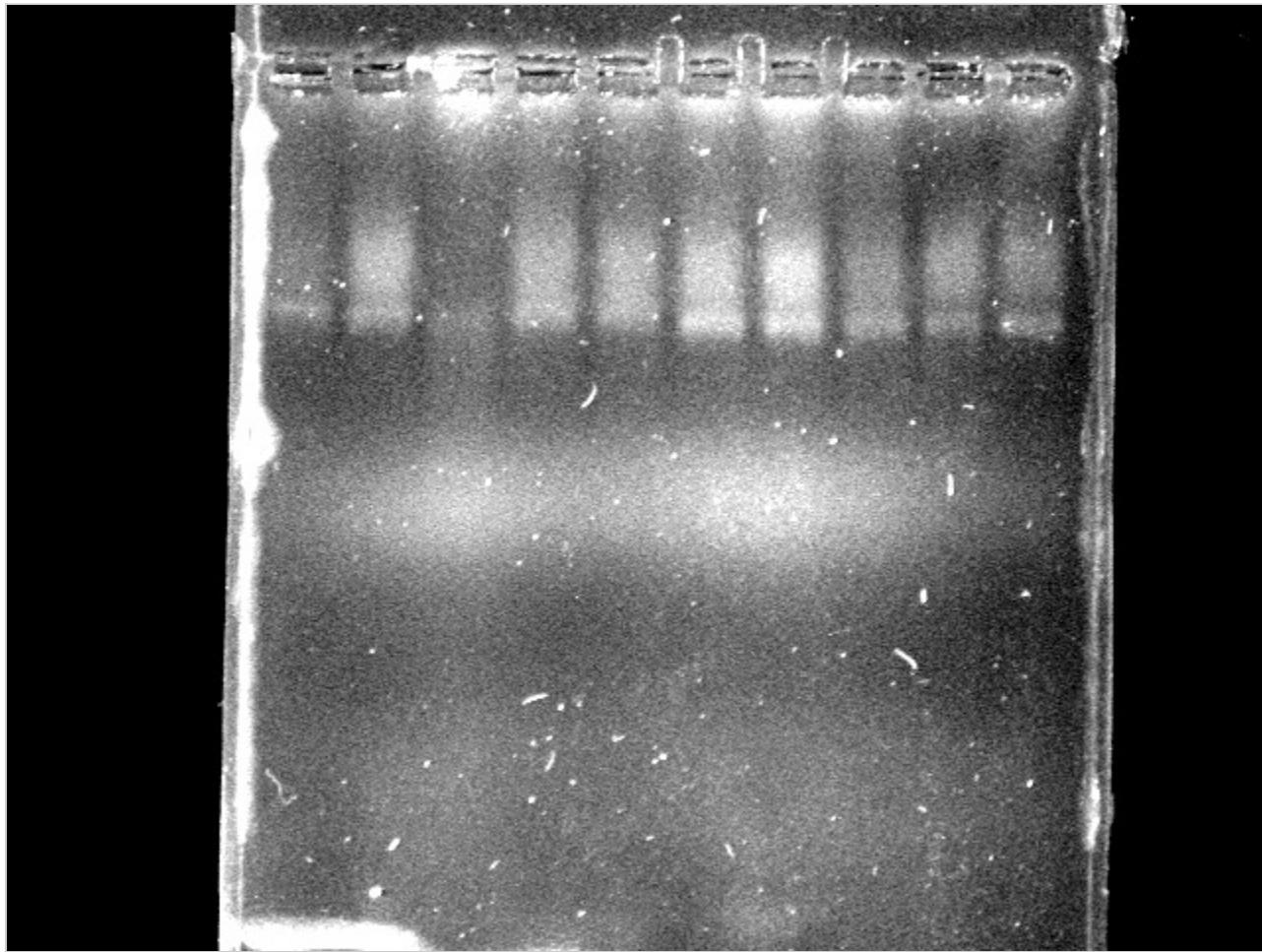
Steps

#	Title	Timer

Experiment Results



Experiment Attached Images



15-08-21_uv_pdg.tif

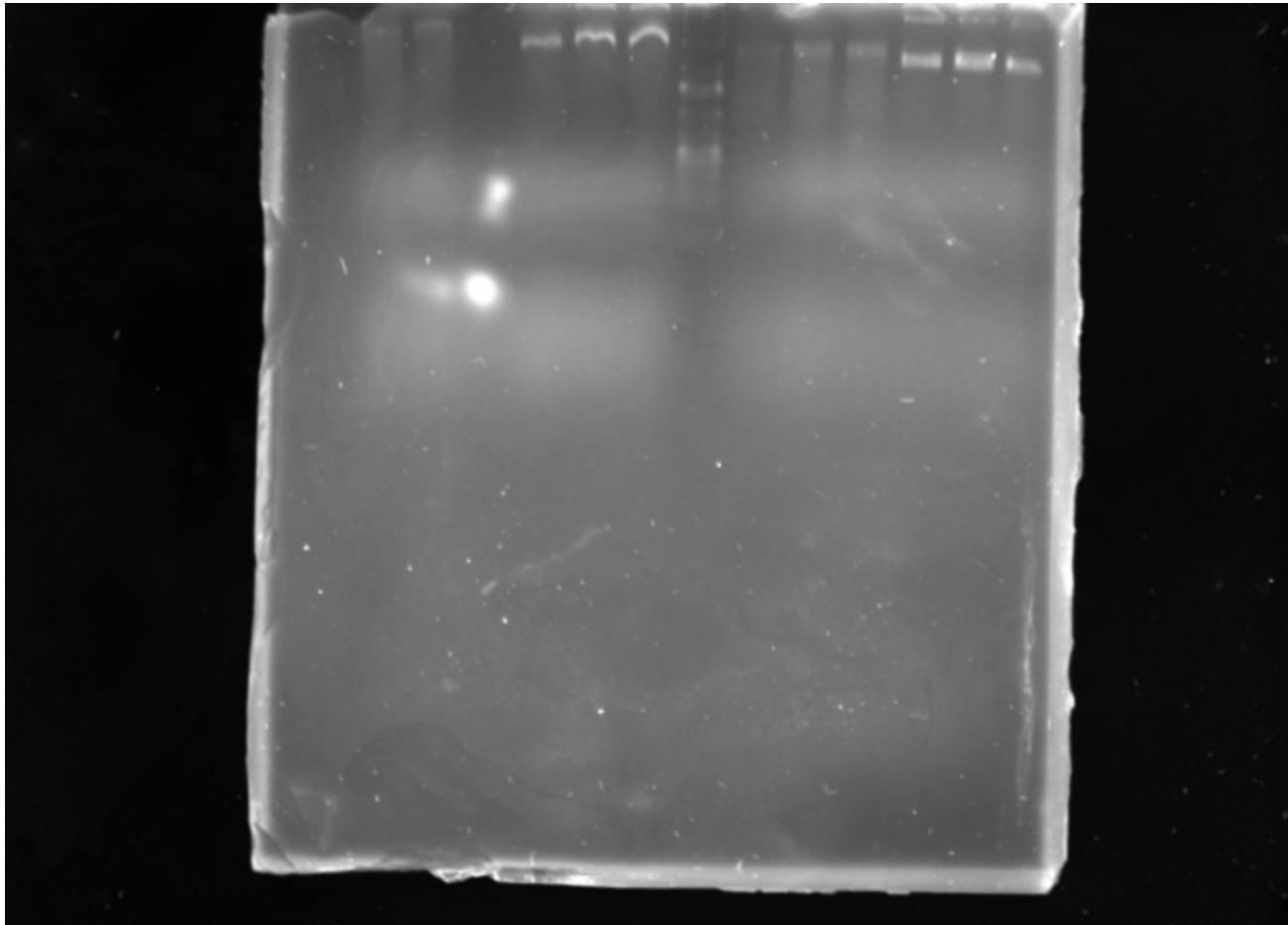
Experiment: 2015-09-12 RFPuv + RFPox alkaline gel

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: RFP uv Experimentation | Owner: Jarrod Shilts

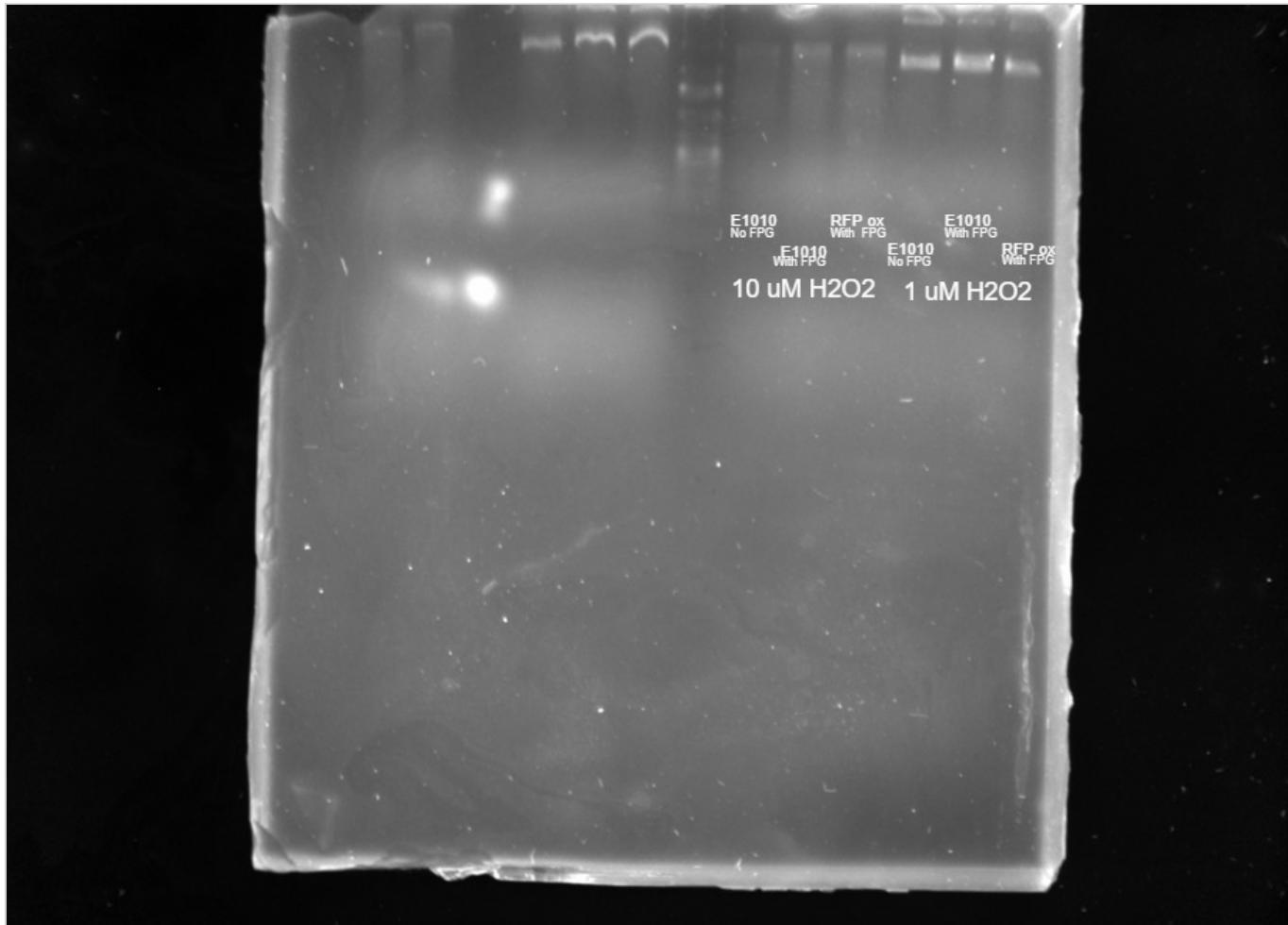
Experiment Procedures

New Procedure

Experiment Results



Experiment Attached Images



15-09-12_uv_ox_plasmid_alkaline.tif

Milestone: gBlock to Biobrick in pSB

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Experiment: 2015-07-21 gBlock PCR

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Signed by Jarrod Shilts on July 25, 2015 at 22:22

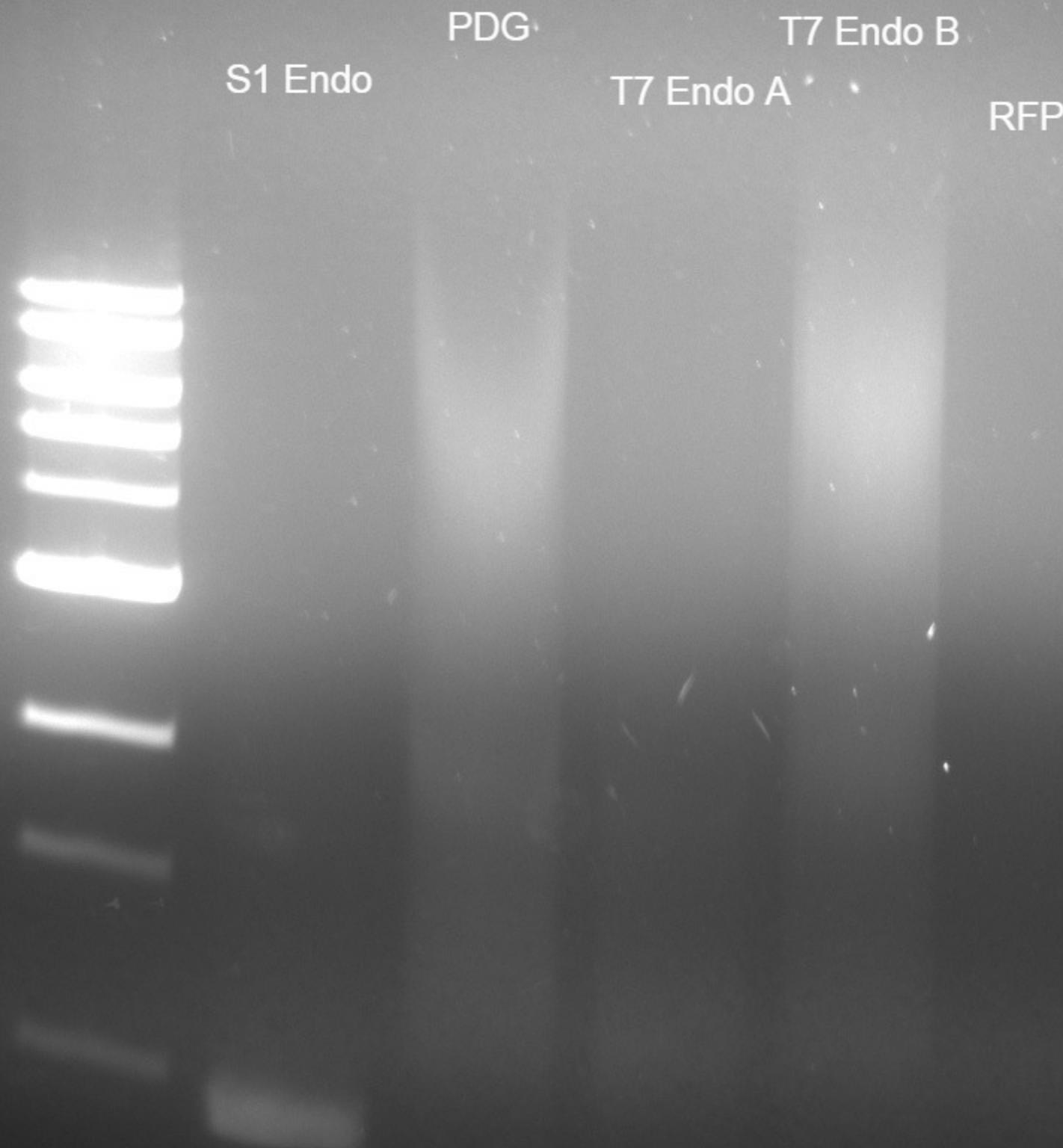
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Resuspend 500 ng of gBlock in 10 ul of ultrapure water. Incubate at 50 degrees 20 min. Parts included: S1 Nuclease, RFP Oxidation Opt, T4 PDG Endonuclease V, T7 Endo I Homology OptA, T7 Endo I Homology Opt B	00:00:00
2	Add 0.5 ul of gBlock to Q5 hot start master mix with Prefix_Bam_FWD and Suffix_Kpn_REV primers. 25 ul reactions for all except RFP Oxidation Opt (50 ul reaction). 98 degrees 30 seconds, 25 cycles of 98 degrees 5 seconds, 66 degrees 10 seconds, 72 degrees 10 seconds, final 2 min 72 degrees.	00:00:00
3	Run 3 ul on gel and image	00:00:00

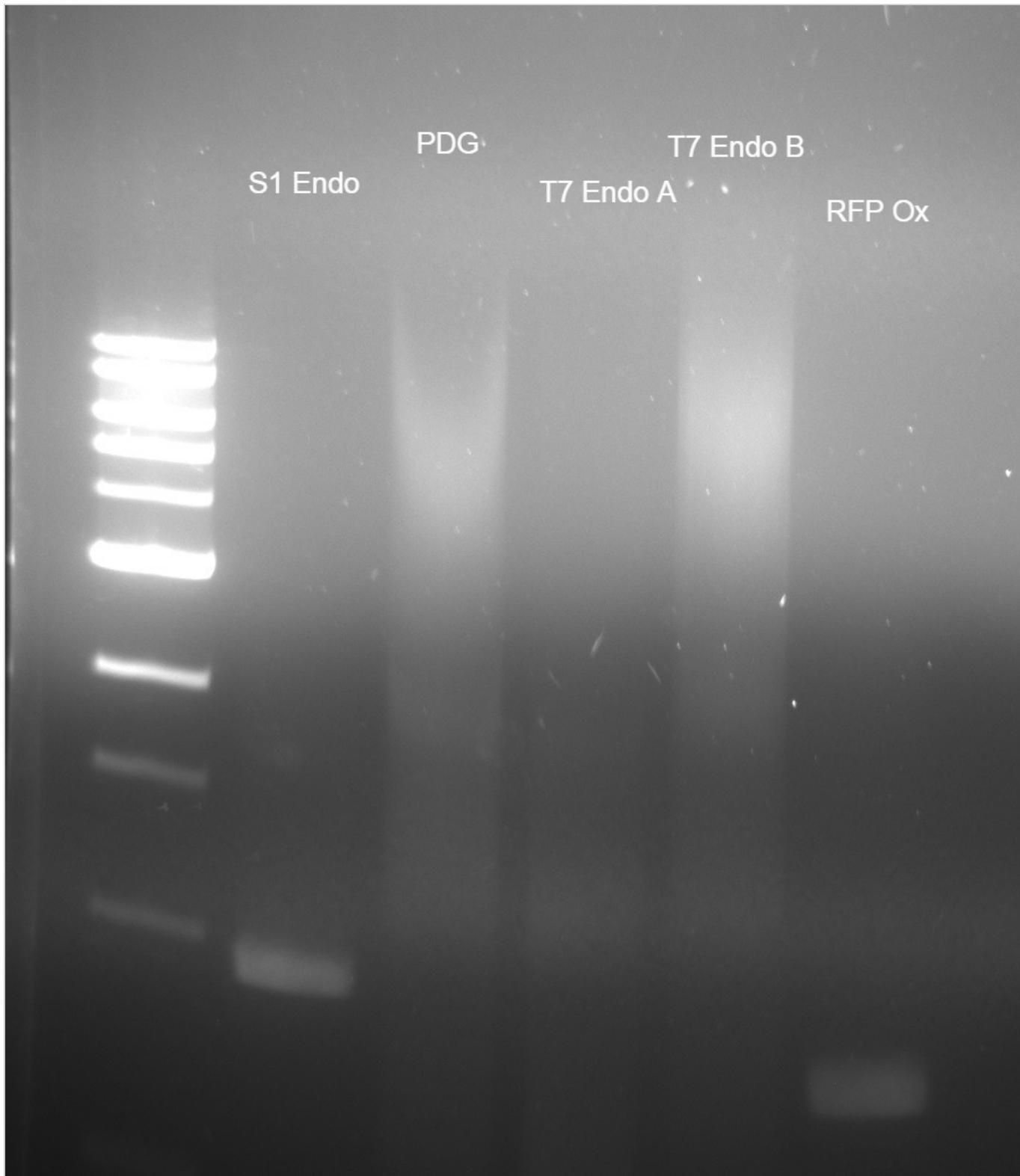
Experiment Results



Experiment Conclusion

Slight amplification on S1 and RFP ox, but no amplification on other gBlocks (likely from palindromic primers). Bands at correct sizes

Experiment Attached Images



7-22-15_gblock_pcr.jpg

Experiment: **2015-07-22 gBlock Ligations**

Signed by Jarrod Shilts on July 25, 2015 at 22:22

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Digest 2 ul of S1 gBlock plus 3 ul of S1 PCR product with EcoRI and PstI in NEBuffer 2.1. Digest 2 ul of RFP ox gBlock plus 3 ul of RFP ox PCR product with EcoRI and PstI in NEBuffer 2.1. Digest 10 ul of pSB1C3 linearized (~600ng) with EcoRI and PstI in NEBuffer 2.1. Incubate 37 degrees 3 hours, 80 degrees 20 min.	00:00:00
2	Set up two ligations for S1 and two for RFP ox with 10 ul of gBlock digest, 3.5 ul of pSB1C3, and rest T4 DNA ligase + buffer + water. Incubate 16 hours at 16 degrees, then store at 4 degrees	00:00:00
3	Digest 3 ul of gBlock for T7 Endo A, T7 Endo B, T4 PDG, K117000 opt, E1010 opt, and 10 ul linerized pSB1C3 with EcoRI and PstI in NEBuffer 2.1 (20 ul digest). Incubate 2 hours 37 degrees, 80 degrees 20 min.	00:00:00
4	Add 4 ul of pSB1C3 digest to the five other digests, plus 3 ul 10x ligation buffer and 2 ul T4 Ligase. Incubate at room temperature for one hour.	00:00:00
5	Transform 5 ul of ligation mixture with 30 sec heat shock, 450 ul SOC, and 1 hour outgrowth.	00:00:00

Experiment Results

All plates produce 1-30 white colonies. In addition, large number of red colonies from residual J04450 in PCR purified backbone.

Experiment: **2015-07-25 gBlock+ pSB1C3 confirmation**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **gBlock to Biobrick in pSB** | Owner: **Jarrod Shilts**

Signed by Jarrod Shilts on July 25, 2015 at 22:34

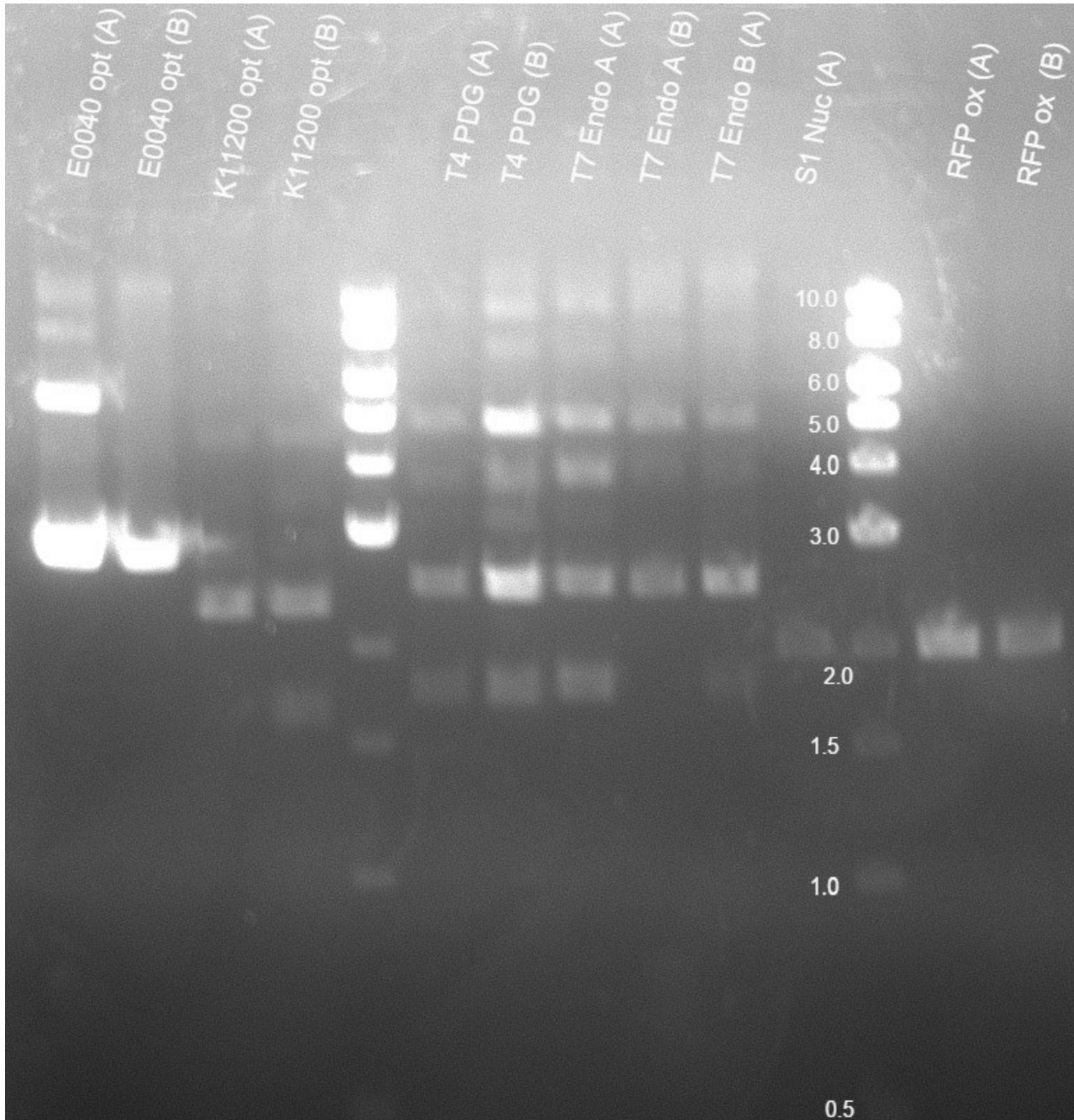
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Make 3 ml liquid cultures of two white colonies per plate. Incubate 15 hours at 37 degrees.	00:00:00
2	Miniprep with viogene kit. Elute in 30 ul. Nanodrop shows go purity but medium-low yield (70-200 ng/ul)	00:00:00
3	Digest 3 ul of plasmid with EcoRI-HF in cutsmart. Incubate 20 minutes at 37 degrees	00:00:00
4	Run on 1% gel at 130 volts	00:00:00

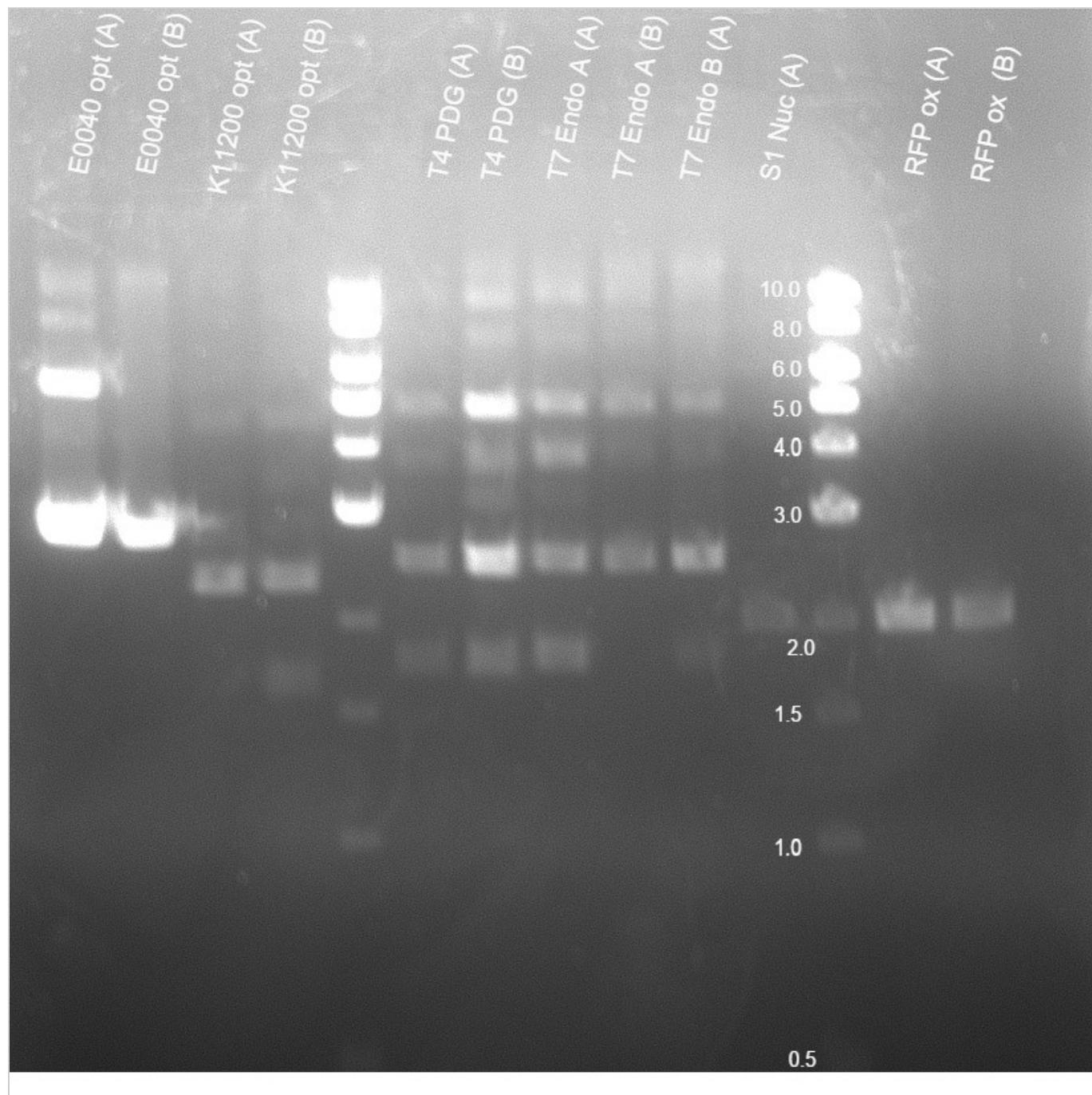
Experiment Results



Experiment Conclusion

Correct sized band for E0040 opt (2.8 kb), T4 PDG (2.5 kb), T7 Endo A (2.5 kb), T7 Endo B (2.5 kb). Incorrect size on K112000 (should be 2.7 kb) and no insert apparent on S1 nuclease and RFP ox

Experiment Attached Images



Experiment: **2015-07-26 Promoter ligations and redo gBlock extractions**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Signed by Jarrod Shilts on July 27, 2015 at 20:48

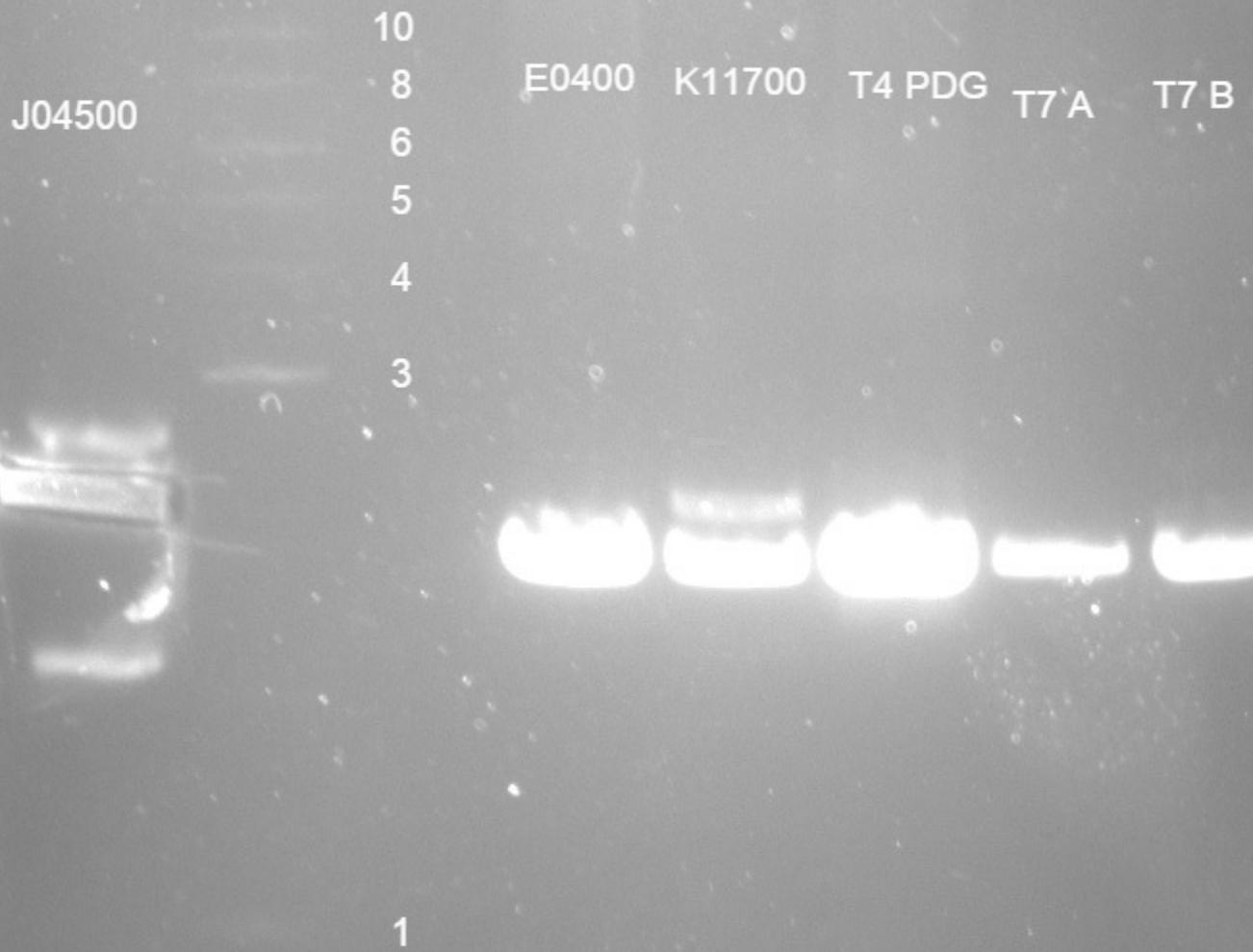
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Digest 2 ul of RFP UV (weighted optimized), RFP ox, and S1 nuclease gBlock in 20 ul digest with EcoRI and PstI.	00:00:00
2	Add 6 ul of EcoRI+PstI digested pSB1C3 from 6/3 to each digest directly. Add 3 ul 10x ligase buffer and 1.1 ul T4 DNA ligase. Incubate 16 hours at 16 degrees	00:00:00
3	Digest 7 ul of miniprep E0400 opt (B), K11700 opt (A), T4 PDG (B), T7 Endo A (B), T7 Endo B (A) with XbaI and PstI in 15 ul reaction. Digest 7 ul of J04500 with SpeI and PstI in a 15 ul reaction	00:00:00
4	Incubate 4 hours at 37 degrees, then 25 min 80 degrees.	00:00:00
5	Run all of the plasmid digests on a 0.8% gel. Cut out bands at correct sizes and gel extract with Qiagen kit	00:00:00
6	Ligate 20 ul gBlock gel extractions with 6 ul J04500 in a 30 ul ligation with T4 ligase. Incubate 12 hours at 16 degrees	00:00:00

Experiment Results

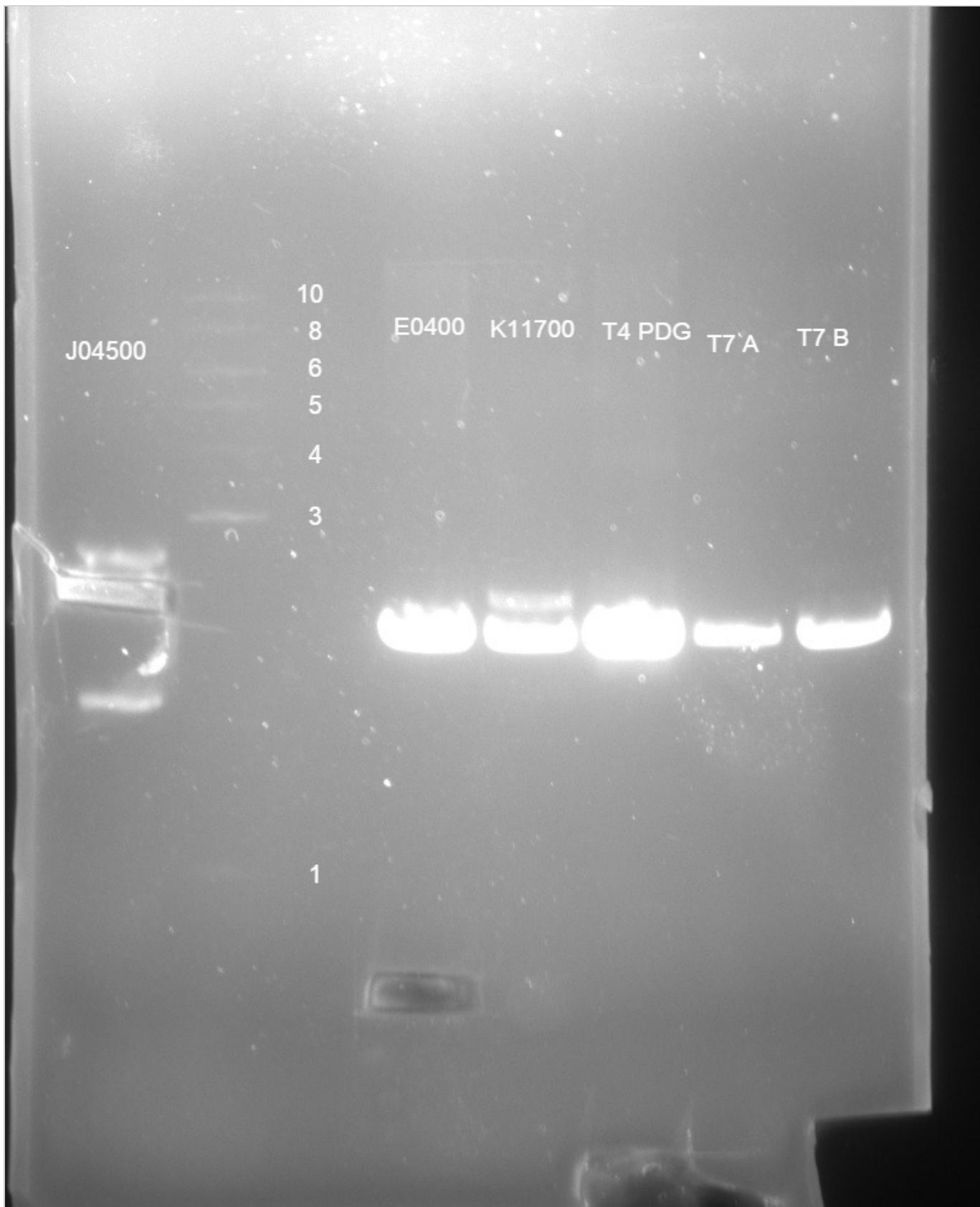


Experiment Conclusion

J04500, E0400 opt, T4 PDG, and T7 Endo B all produce insert bands at correct size. Other plasmids do not produce visible bands below 1 kb.

Upon transformation, all plates produce colonies. RFP UV, RFP ox, and S1 produce >100 colonies (with <10 red background colonies). Promoter ligations produce 1-2 colonies, or none in the case of E0400

Experiment Attached Images



150726_gblock.jpg

Experiment: **2015-07-27 ligation transformations**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Signed by Jarrod Shilts on July 28, 2015 at 21:14

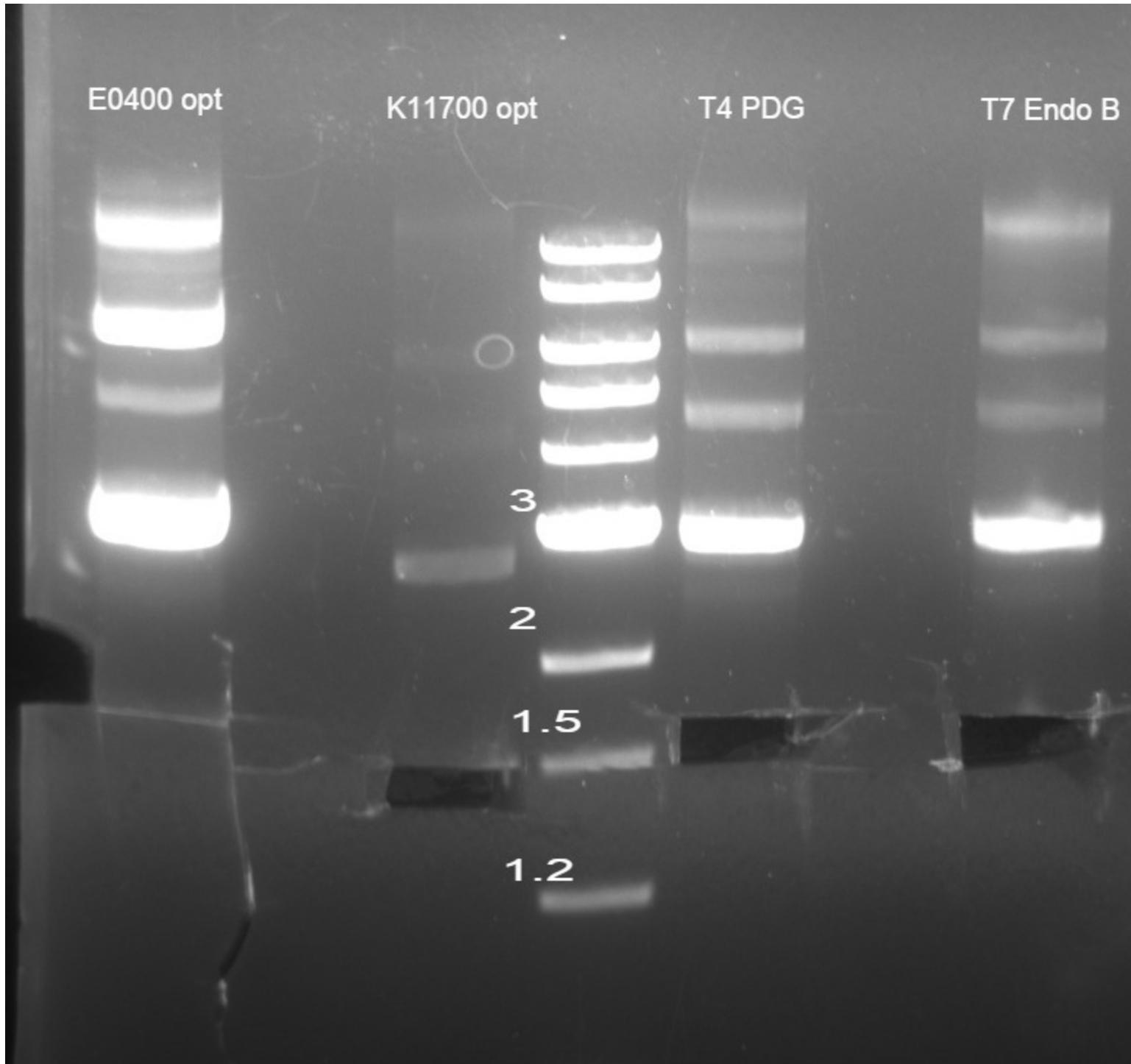
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Load 10 ul of minipreped E0400 opt (B), K11700 opt (A), T4 PDG (B), T7 Endo B (A) on 0.7% gel	00:00:00
2	Gel extract with Viogene kit. Elute in 30 ul. Yield of ng/ul	00:00:00
3	Transform 5 ul of ligation mixture from 7/26 or 3 ul of gel extracted plasmid into BL21. Heat shock 30 seconds, 1 hour outgrowth.	00:00:00

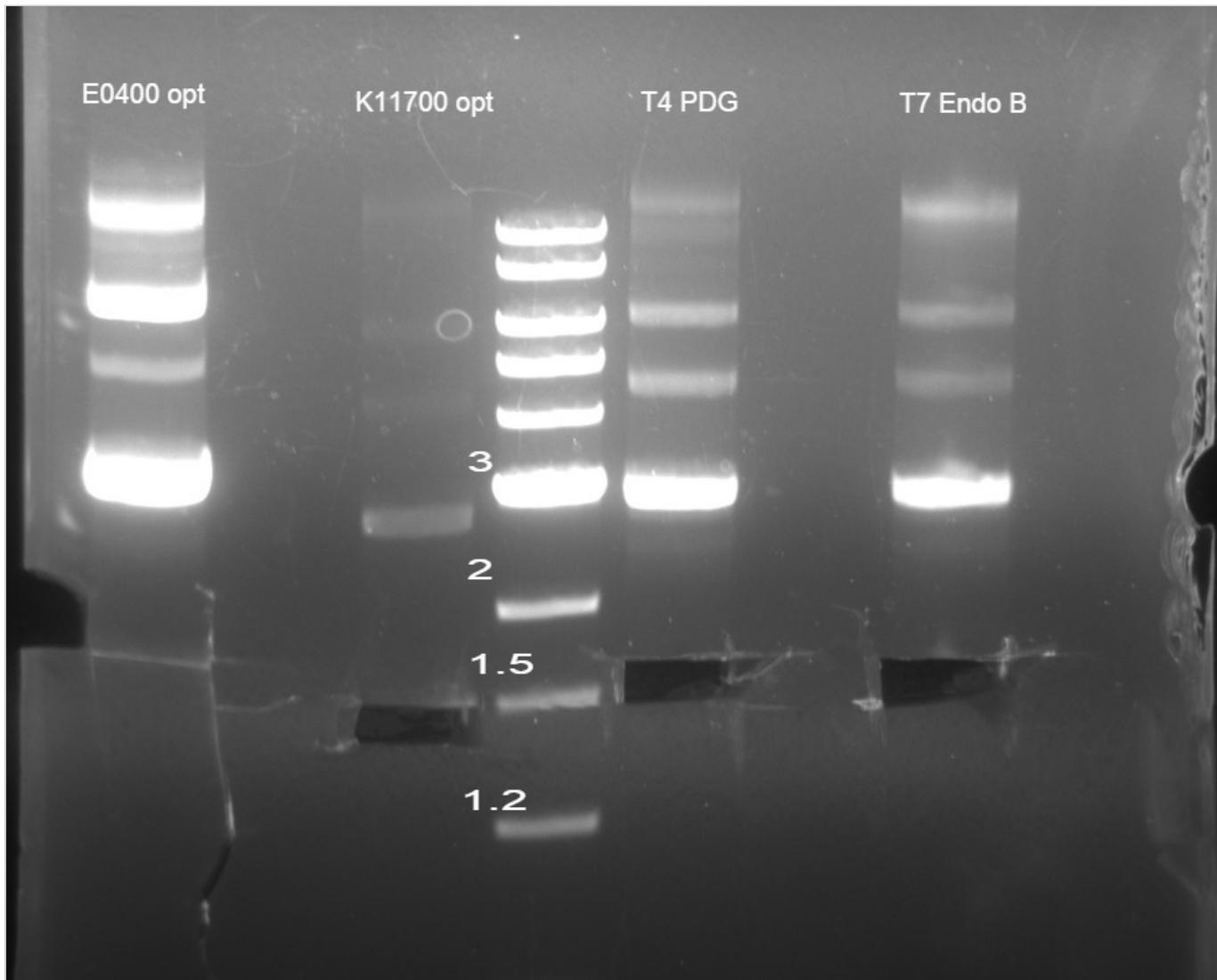
Experiment Results



Experiment Conclusion

No colonies after 18 hours on plates except for T7 Endo B (2 colonies).

Experiment Attached Images



150727_gblock.jpg

Experiment: 2015-07-30 ligation confirmation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Digest 3 ul of plasmids from 7/29 miniprep with EcoRI in cutsmart buffer (20 ul digest). Incubate at 37 degrees for one hour	00:00:00
2	Add 5 ul loading dye and load 11 ul on to 1% gel with 7 ul of KB ladder	00:00:00
3	Send samples for GeneWiz sequencing with VF2 primer	00:00:00

Experiment Results

J04500+E0400opt

J04500+T4PDG

J04500+T7 Endo B

S1 (A)

S1 (B)

T7 Endo B



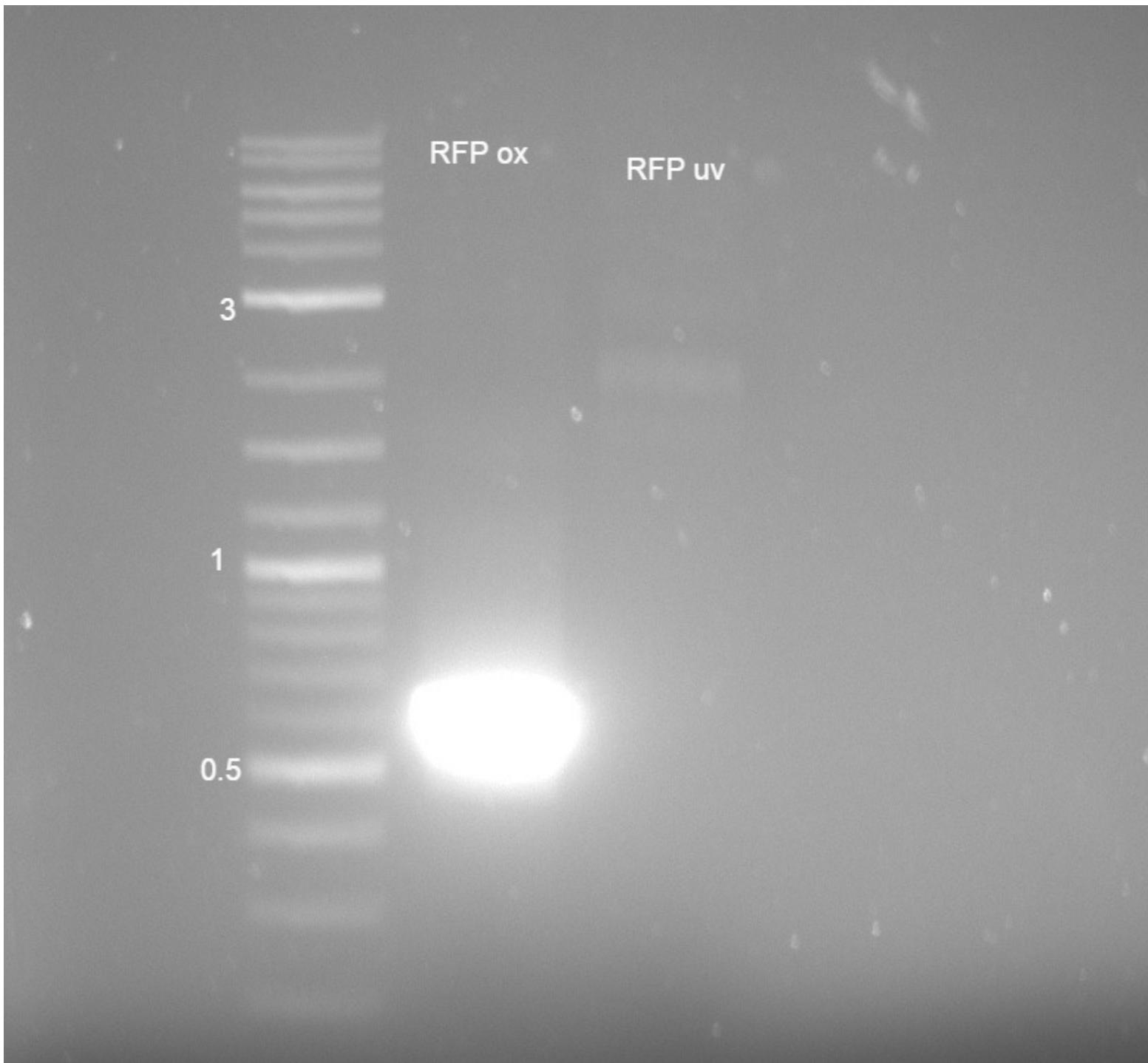
RFP ox (A)

RFP ox (B)

RFP ox (C)

RFP UV (A)

RFP uv (B)



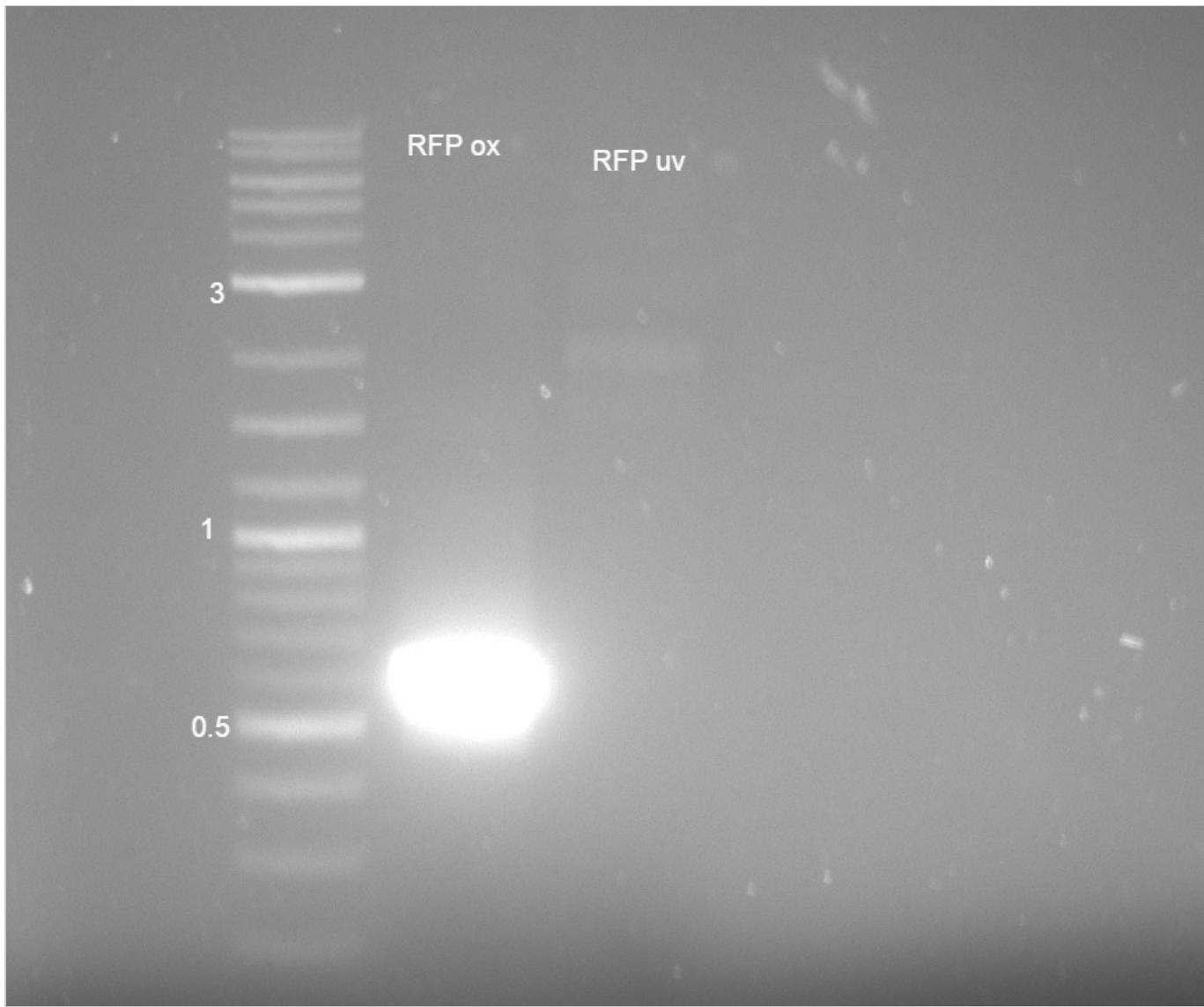
Experiment Conclusion

Insufficient amount of EtBr and or sample added. Approximately correct size on promoter assemblies, although resolution insufficient to determine if J04500 in place. RFP ox also at approximately correct size (2.7)

PCR of RFP gives either too small product (RFP ox at ~650bp instead of 1000) or too large (RFP uv above 2000)

Experiment Attached Images

150730_conf.png



150801_pcr_rfp.jpg

Experiment: **2015-08-18 RFP ox, S1, pKIKO confirmation**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Signed by Jarrod Shilts on August 22, 2015 at 15:58

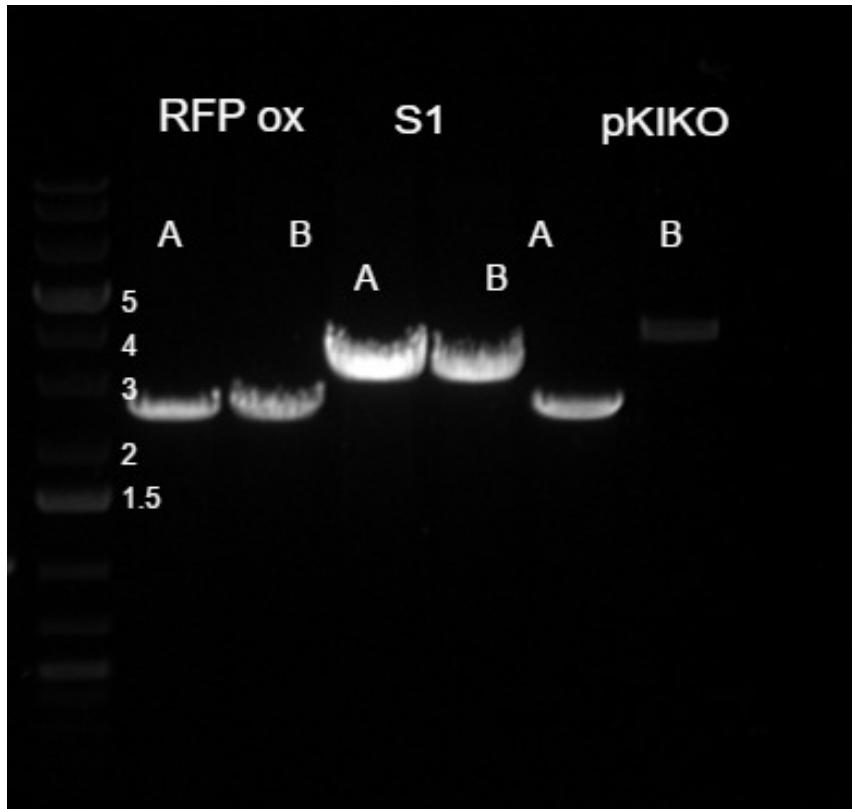
Experiment Procedures

New Procedure

Steps

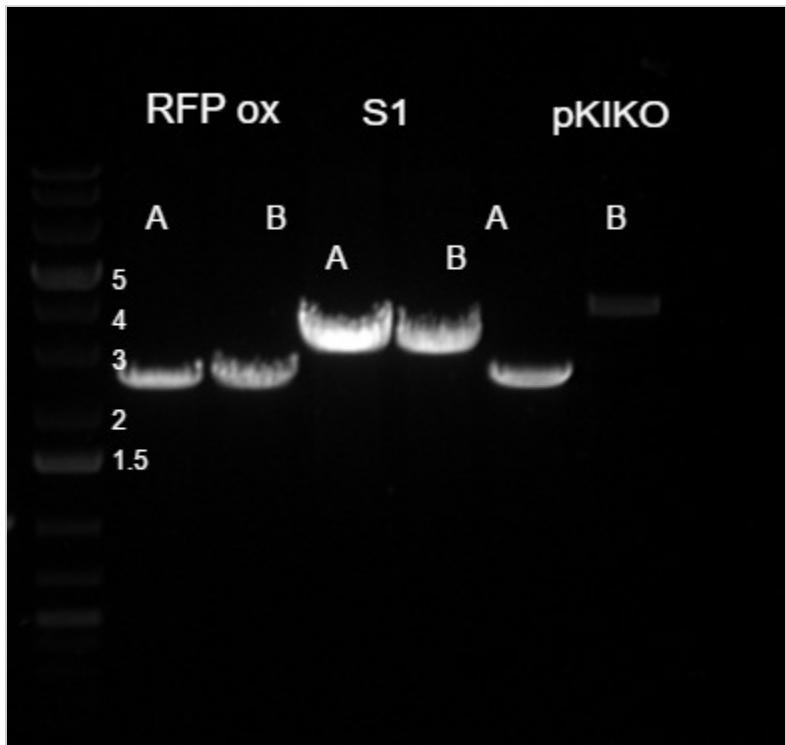
#	Title	Timer
1	Streak bacterial stab of pKIKO ArsB Cmr onto chlor plates. After 24 hours, grow two colonies in LB with chlor and kan antibiotic	00:00:00
2	Miniprep pKIKO cultures and ligations for RFP ox and S1 into pSB1C3	00:00:00
3	Diagnostic digest with EcoRI on 4 ul of miniprep DNA. Run on 0.8% gel	00:00:00

Experiment Results



Correct sizes on both RFP ox and S1. pKIKO B approximately corect

Experiment Attached Images



15-08-19_gBlock.png

Experiment: 2015-08-19 Biobrick sequencing

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

Signed by Jarrod Shilts on August 20, 2015 at 22:47

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 5 ul of 5 uM primer (VF2 or VR) to approx 500 ng of plasmid DNA, in a total volume of 15 ul. Note that most minipreps of extremely poor yield (<5 ng/ul). Submit 16 orders to GeneWiz	00:00:00
2	Re-transform 2 ul of each miniprep in BL21. 30 sec heat shock, plate 100 ul of 400 ul outgrowth	00:00:00
3	Samples: E0040 opt (B), K117000 opt (A), T4 (B), T7 A (B), T7 B (A) from July. RFP uv (C), S1 (A), RFP ox (A) from August.	00:00:00

Experiment Results

No transformations other than T7 A produce any colonies. Sequence confirmation of successful assembly of RFP uv, RFP ox, S1 nuclease, and possibly T7 B. Others inconclusive, not indications of failed assemblies.

Experiment: **2015-08-22 Re-acquiring assemblies**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **gBlock to Biobrick in pSB** | Owner: **Jarrod Shilts**

Experiment Procedures

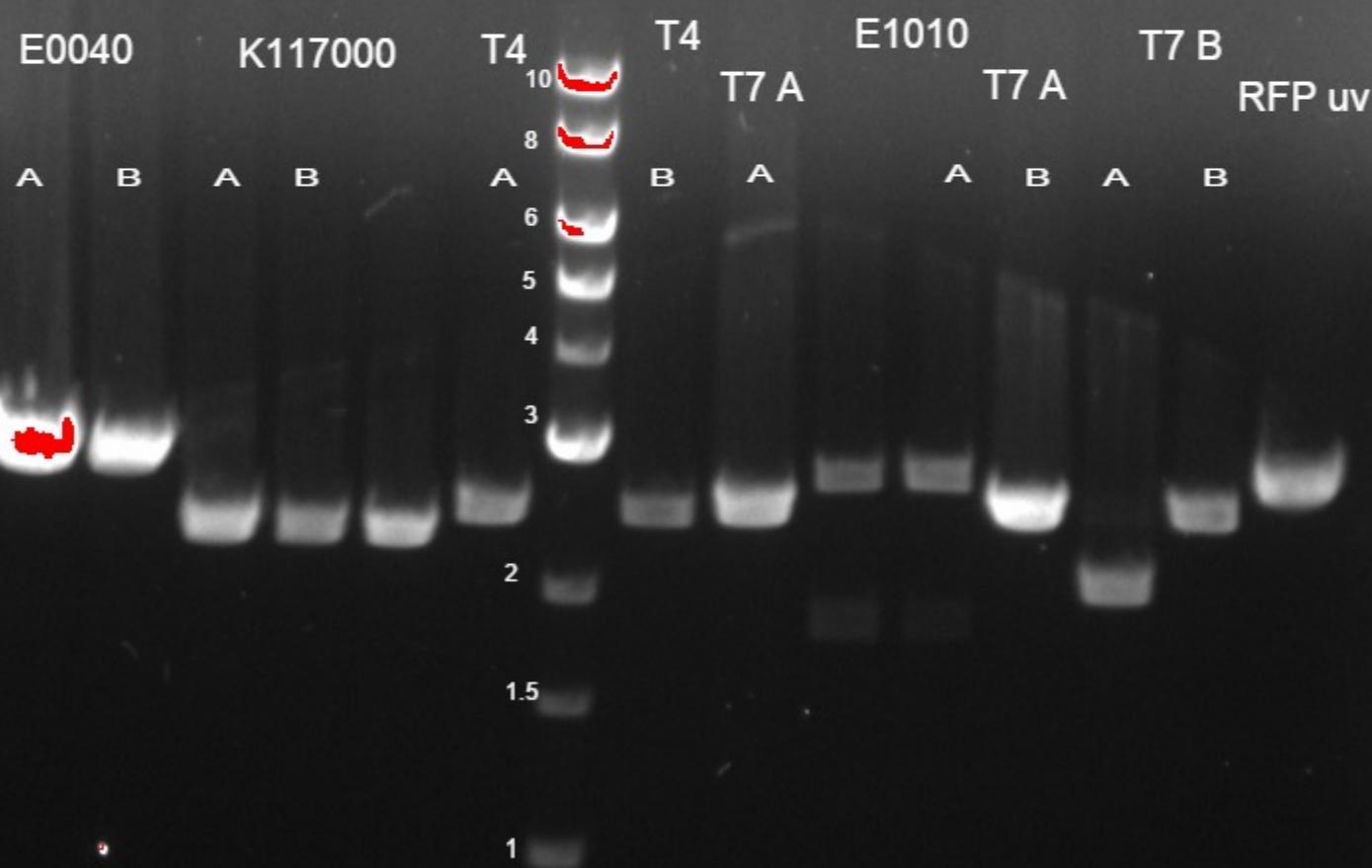
New Procedure

Steps

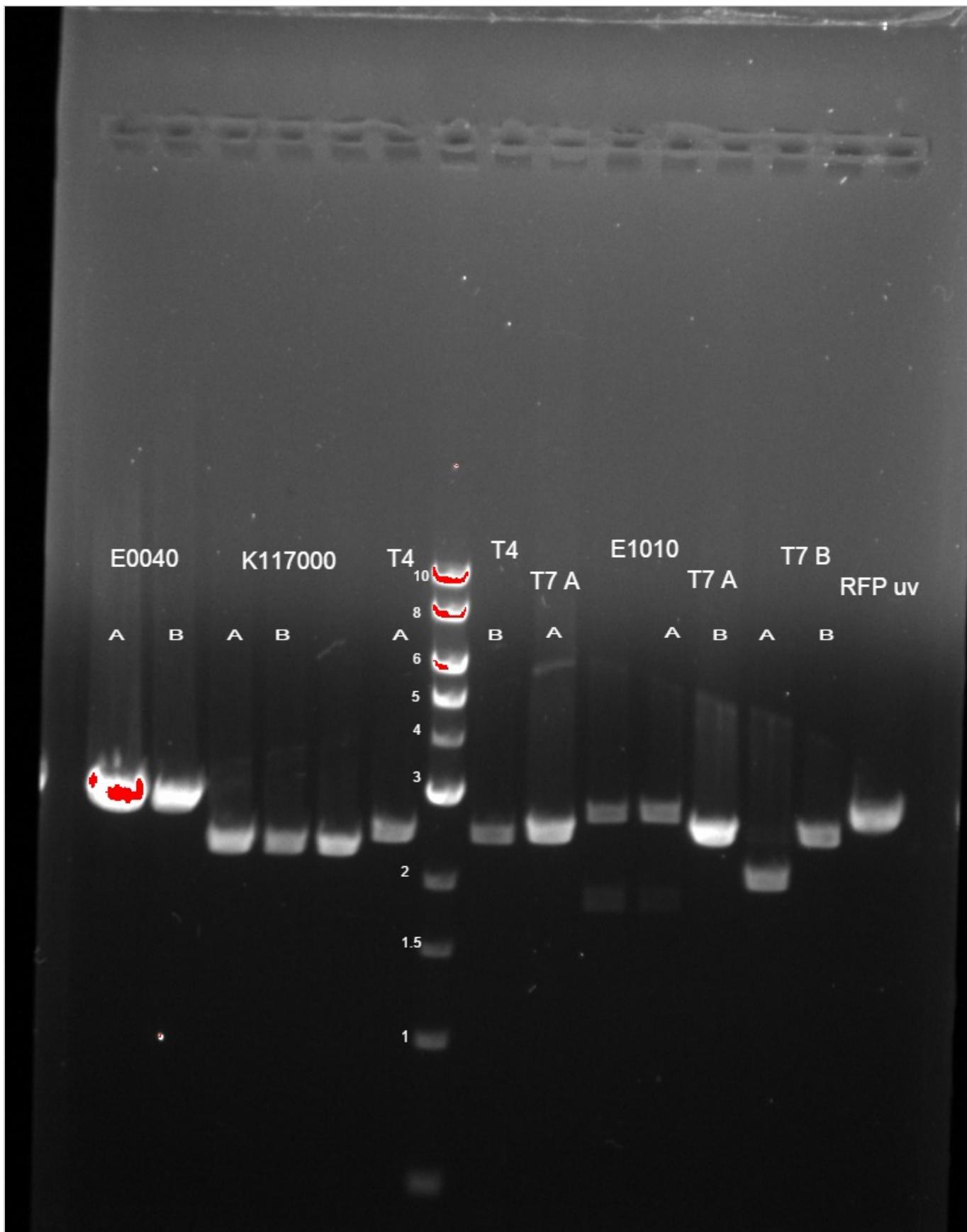
#	Title	Timer
1	Transform into BL21 2 ul of dilute, low-quality miniprep of E0040 opt (B), K117000 opt (A), T4 (B), T7 A (B), T7 B (A) from July. RFP uv (C), S1 (A), RFP ox (A)	00:00:00
2	Make at least two 5 ml cultures of each and incubate 20 hours	00:00:00
3	Miniprep, eluting in 50 ul. All concentrations >200 ng/ul with good purity.	00:00:00
4	Digest 2 ul of miniprep with EcoRI and run on 1% gel	00:00:00

Experiment Results

All sizes on gBlock parts seem correct except T7 B (A). E1010, both E1010a and E1010 from 3/25 give second, faint band below expected 2.7



Experiment Attached Images



Experiment: **2015-08-29 Ligation confirmation**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: gBlock to Biobrick in pSB | Owner: Jarrod Shilts

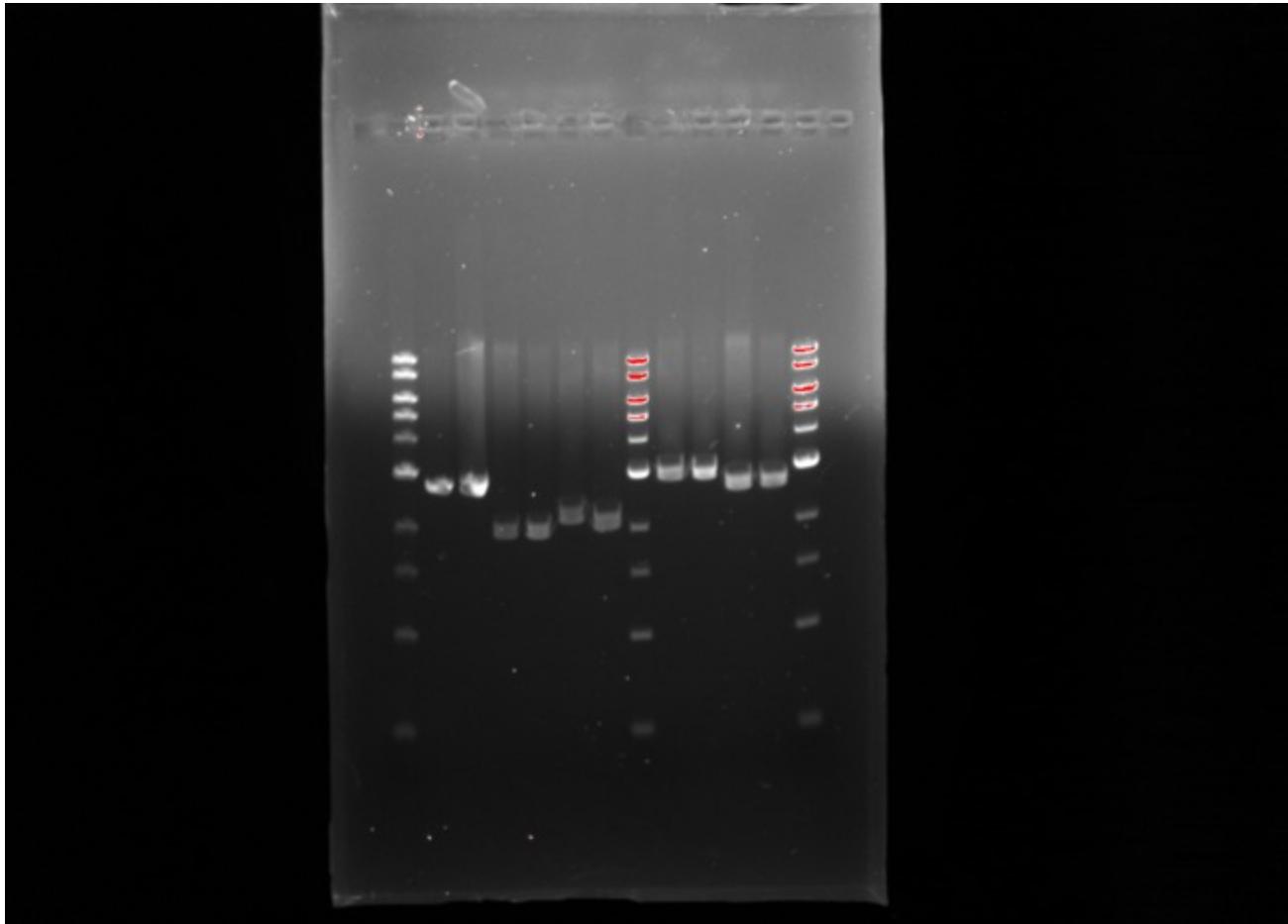
Experiment Procedures

New Procedure

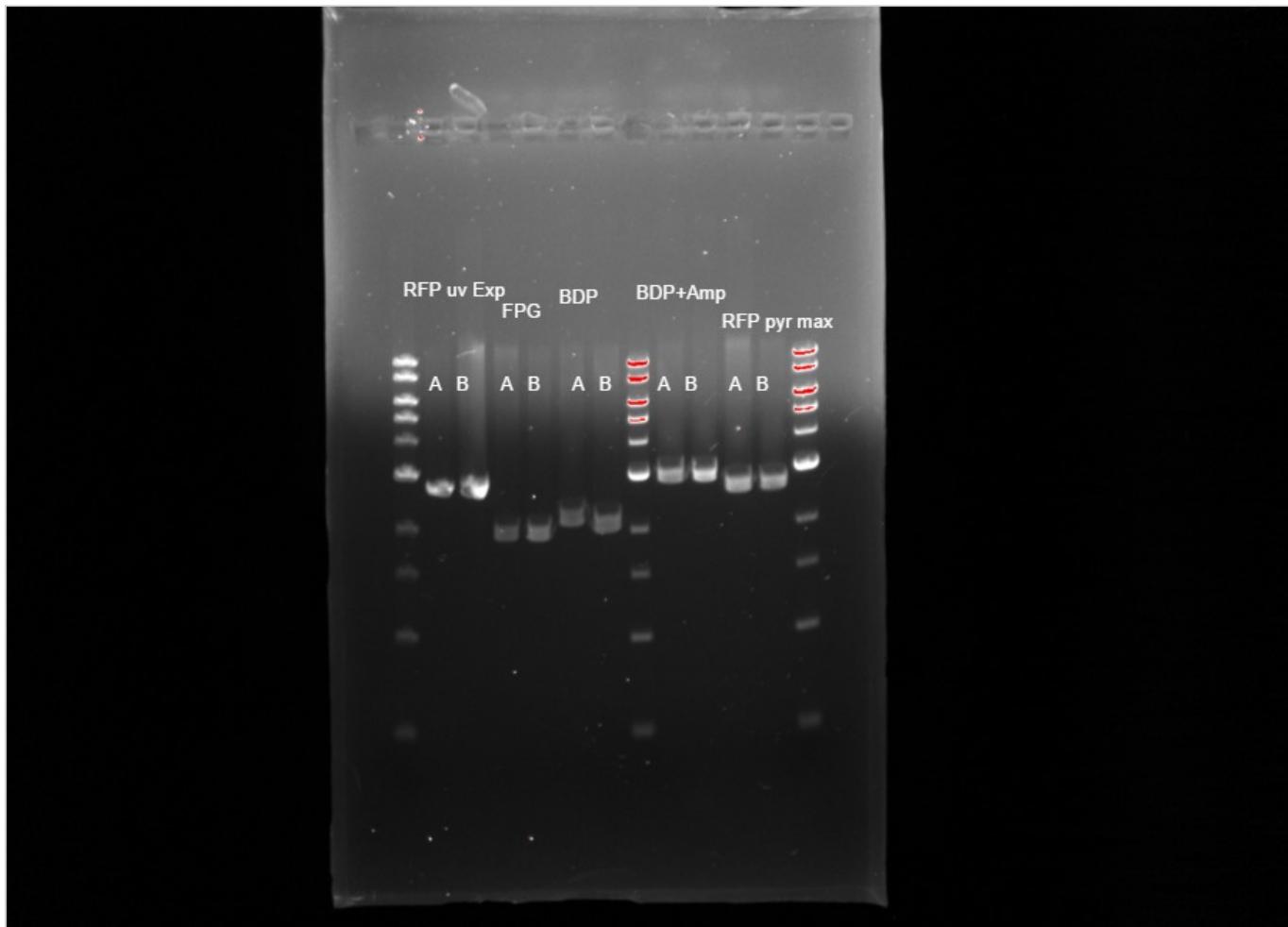
Steps

#	Title	Timer
1	Miniprep cell cultures with 50 ul elution	00:00:00
2	Digest for 45 min in 10 ul reaction with 0.5 ul EcoRI	00:00:00
3	Run all 10 ul on 1% gel, 130 V 45 min	00:00:00

Experiment Results



Experiment Attached Images



15-08-29_ligation_conf.tif

Milestone: Ura3 Recombination

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Owner: Jarrod Shilts

Due Date: 2015-08-16

Experiment: 2015-08-07 - Genomic DNA Extraction

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Ura3 Recombination
| Owner: Stephen Lee

Signed by Stephen Lee on August 09, 2015 at 23:48

Smash and grab genomic DNA extraction of yeast strain 199a, a mating strain with wild-type URA3.

Experiment Procedures

Smash and Grab Genomic DNA Extraction

From Aug 07, 2015 15:00

Yeast (*S. cerevisiae*) mating strains 199a and 200a were grown overnight in YPD before undergoing a *smash and grab* genomic DNA extraction.

Steps

#	Title	Timer
1	Pellet cells from up to 3mL of overnight culture in a 1.5mL microcentrifuge tube (aspirate excess media after each addition of culture).	00:00:00
2	Resuspend cell pellet in 0.2mL of <i>smash and grab</i> lysis buffer.	00:00:00
3	Add 0.3g (roughly 0.3mL) of glass beads using a plastic funnel.	00:00:00
4	Add 0.2mL of a 1:1 mix of phenol and chloroform (with Tris buffer added, pH 8.0).	00:00:00
5	Vortex at top speed for 2min total (4 x 30 second bursts with 15 seconds on ice between each).	00:00:00
6	Add 0.2mL of TE (10mM Tris, 1 mM EDTA, pH 8.0) and vortex again for a few seconds.	00:00:00
7	Spin for 5min at top speed in a microcentrifuge.	00:00:00
8	Immediately transfer the aqueous (upper) phase to a fresh microcentrifuge tube.	00:00:00
9	Add 2 volumes of absolute ethanol at room temperature. Mix thoroughly.	00:00:00
10	Spin for 15min at top speed in a microcentrifuge.	00:00:00

11	Aspirate the supernatant.	00:00:00
12	Rinse the DNA pellet with 0.5mL of cold, 70% ethanol. Push any DNA adhered to the sides of the tube to the bottom before proceeding.	00:00:00
13	Spin for 5min at top speed in a microcentrifuge.	00:00:00
14	Aspirate the supernatant.	00:00:00
15	Vacuum dry pellet for 15-30min to remove excess ethanol.	00:00:00
16	Resuspend DNA in 30uL TE.	00:00:00

Experiment Results

Genomic DNA extraction was successful. Gel confirmation of amplification off 199a genomic DNA can be found under "[pUC19/URA3 Insert Digestion with BamHI-HF, SpeI](#)".

Experiment: 2015-08-09 - PCR Amplification of URA3 Insert

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Ura3 Recombination
| Owner: Stephen Lee

Signed by Stephen Lee on August 10, 2015 at 16:15

PCR amplification of URA3 with flanking DNA using Platinum Taq polymerase and Ura3_Spe_Ext primers (F and R).

Ingredients

- 0.2 μ L Platinum Taq Polymerase
- 5 μ L Platinum Taq Buffer
- 1.5 μ L MgCl₂
- 1 μ L dNTPs
- 1 μ L Ura3_Spe_Ext Forward Primer
- 1 μ L Ura3_Spe_Ext Reverse Primer
- 1 μ L 199a URA3 Template DNA
- 39.3 μ L ddH₂O

Conditions

- 55° annealing temperature
- 72° extension temperature
- 3:00 extension time
- 35 cycles
- Held at 12°

Experiment Procedures

Primer Dilution

Steps

#	Title	Timer
1	Centrifuge tube to collect DNA pellet	00:00:00
2	Add 10x μ L ddH ₂ O to DNA in primer tube to create 100 μ M primer stock solution (x = nanomolar quantity)	00:00:00
3	Use pipetting to resuspend DNA	00:00:00
4	Add 90 μ L of ddH ₂ O to a microcentrifuge tube labeled with primer name and concentration (10 μ M)	00:00:00
5	Add 10 μ L of primer stock solution for 100 μ L total volume (10 μ M primer working solution)	00:00:00

Preparation

Steps

#	Title	Timer
1	Prepare master solution by combining the following in a microcentrifuge tube:	00:00:00
2		00:00:00
3		00:00:00

Ingredient	Volume (1x)	Volume (2x)	Volume (4x)	Volume (8x)	Volume (10x)
Platinum Taq Polymerase*	0.2µL	0.4µL	0.8µL	1.6µL	2µL
Platinum Taq Buffer	5µL	10µL	20µL	40µL	50µL
MgCl ₂	1.5µL	3µL	6µL	12µL	15µL
dNTPs	1µL	2µL	4µL	8µL	10µL
5' Forward Primer	1µL	2µL	4µL	8µL	10µL
3' Reverse Primer	1µL	2µL	4µL	8µL	10µL
Template DNA	1µL	2µL	4µL	8µL	10µL
ddH ₂ O	39.3µL	78.6µL	157.2µL	314.4µL	393µL
Total	50µL	100µL	150µL	200µL	250µL

*Add Platinum Taq Polymerase last

Running PCR

Steps

#	Title	Timer
1	Fill each properly labeled PCR tube with 49µL of Master Mix and 1 µL of template DNA	00:00:00
2	Place PCR tubes in the thermocycler and select the appropriate program	00:00:00
3	Edit the program for the correct extension time (1 min/kbp) and annealing temperature	00:00:00
4	Run the program and hold samples at 12° if not removed immediately	00:00:00

Experiment Results

PCR was successful. Gel confirmation can be found under "[pUC19/URA3 Insert Digestion with BamHI-HF, SpeI](#)".

Linked Resources

- Polymerase Chain Reaction (NEB) (Protocol)
- Polymerase Chain Reaction (Invitrogen) (Protocol)

Experiment: 2015-08-10 - pUC19/URA3 Insert Digestion with BamHI-HF, SpeI

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Ura3 Recombination
| Owner: Stephen Lee

Signed by Stephen Lee on August 10, 2015 at 16:28

Digestion of pUC19 vector and 199a URA3 insert fragments with BamHI-HF and SpeI.

NOTE: pUC19 should have been digested with BamHI and **XbaI**. The BamHI/SpeI digested vector was redigested with XbaI following gel imaging (below) and prior to phosphatase treatment and ligation with the URA3 insert.

Ingredients

- CutSmart Buffer
- BamHI-HF
- SpeI
- pUC19 or 199a URA3 insert
- ddH₂O

Experiment Procedures

Preparation

Steps

#	Title	Timer
1	Prepare master solution by combining the following in a PCR tube:	00:00:00

Ingredient	Volume
10X Buffer (with BSA added)	10µL
DNA	10µL (1-3µg)
Restriction enzyme 1	1µL
Restriction enzyme 2 (if needed)	1µL
ddH ₂ O	to 100µL
Total	100µL

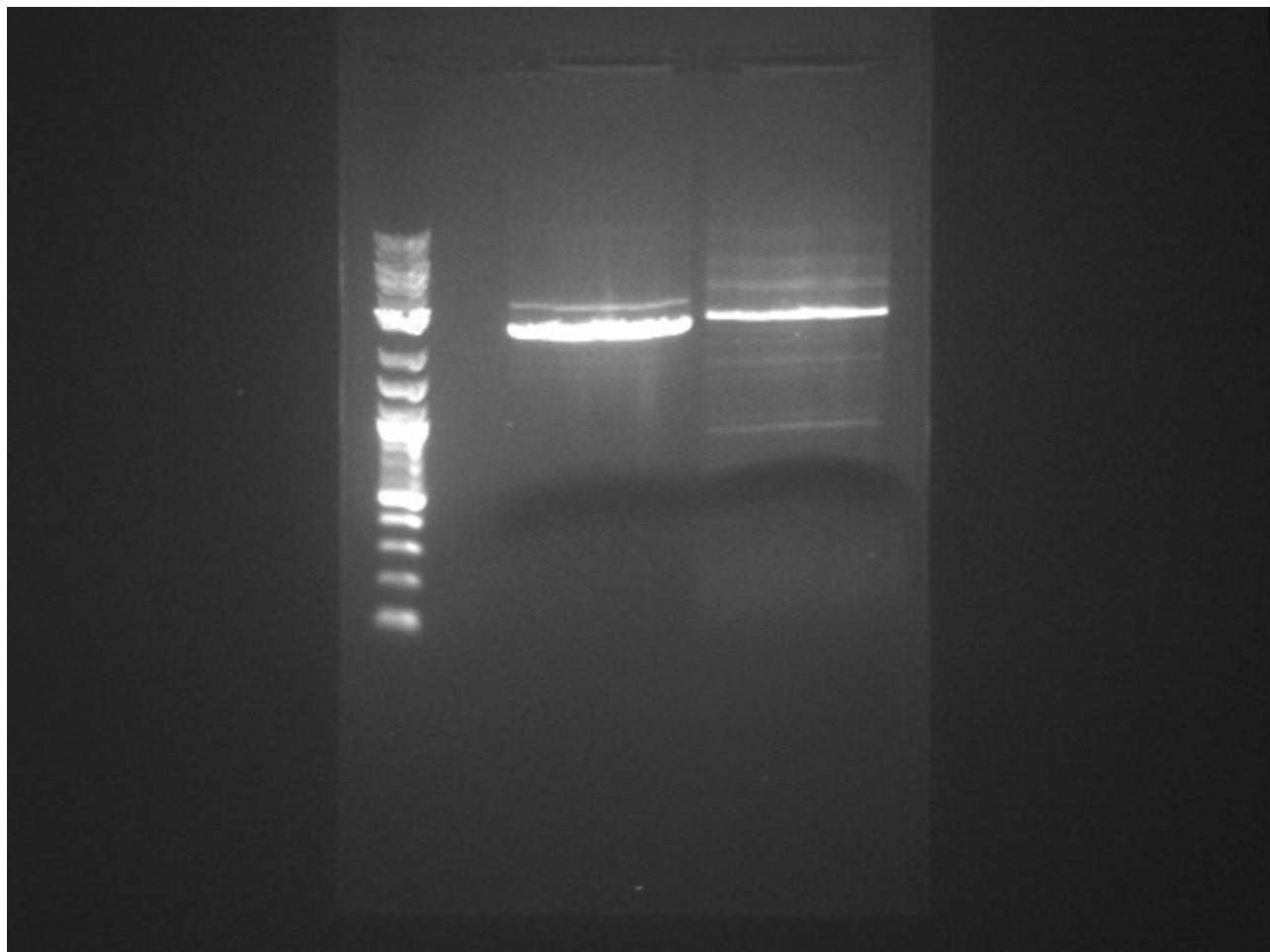
Incubation & Isolation

Steps

#	Title	Timer
1	Incubate at 37°C for 240 min	00:60:00
2	Optional: Heat inactivate at 65°C for 15 minutes or at 80°C for 20 minutes (see chart for proper temperature)	00:15:00
3	Run 0.7% agarose gel to isolate vector from insert and use gel extraction if DNA needs to be recovered	00:00:00

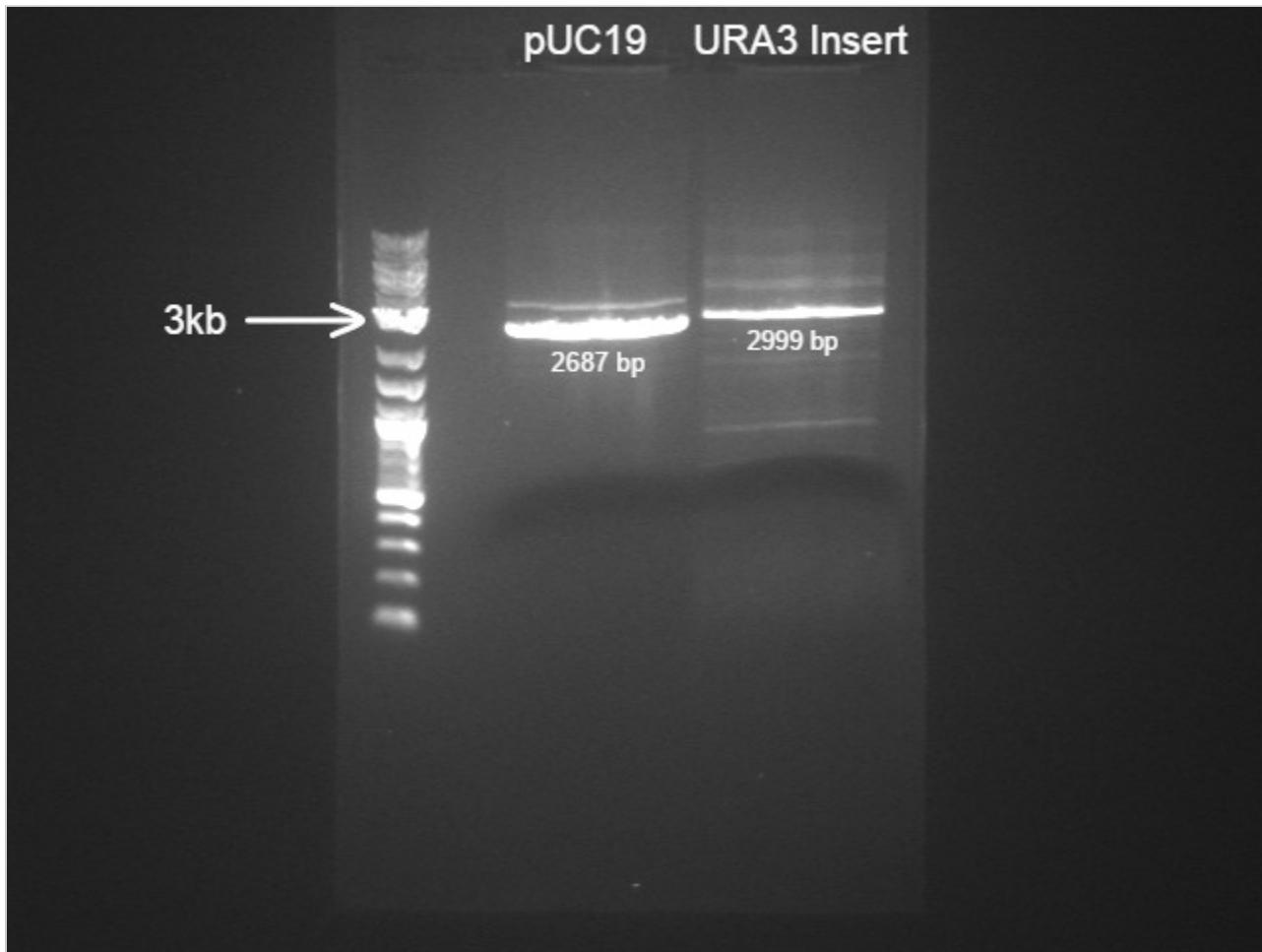
Tip: Incubate for 4-6 hours if using synthesized DNA or PCR products

Experiment Results



Digestion appears successful.

Experiment Attached Images



pUC19_URA3_BamHI_SpeI_8-10-15.tif

Linked Resources

- Restriction Enzyme Digest (Protocol)

Experiment: **2015-08-11 - Gel Extraction of pUC19/URA3 Digest**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Ura3 Recombination**
| Owner: **Stephen Lee**

Purpose: Remove DNA from agarose gel for reuse

Ingredients

- Agarose Gel
- QIAquick Gel Extraction Kit

- ddH₂O @ 65°C

Experiment Procedures

Gel Extraction

Steps

#	Title	Timer
1	Excise the DNA fragment from the agarose gel with a clean, sharp scalpel while minimizing excess gel	00:00:00
2	Weigh the gel slice in a colorless tube and add 3 volumes Buffer QG to 1 volume of gel (100 mg ~ 100 µl) [For >2% agarose gels, add 6 volumes Buffer QG]	00:00:00
3	Incubate at 50°C for 10 min (or until the gel slice has completely dissolved) and vortex the tube every 2–3 min to help dissolve gel	00:10:00
4	After the gel slice has dissolved completely, check that the color of the mixture is yellow	00:00:00
5	If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix and the color of the mixture will turn yellow	00:00:00
6	Add 1 gel volume of isopropanol to the sample and mix	00:00:00
7	Place a QIAquick spin column in a provided 2 ml collection tube and apply the sample to the column	00:00:00
8	Incubate for 2 mins and then centrifuge at 13,000 rpm for 1 min	00:03:00
9	Discard flow-through (for volumes >800 µl repeat until entire sample has been spun)	00:00:00
10	Add 0.75 ml Buffer PE to the column and let incubate for 1 min	00:01:00
11	Centrifuge at 13,000 rpm for 1 min and then discard flow-through	00:01:00

12	Centrifuge at 13,000 rpm for 3 min and place column in clean, labeled microcentrifuge tube	00:03:00
13	Add 50 µl of ddH ₂ O at 65°C and incubate for 5 mins	00:05:00
14	Centrifuge at 13,000 rpm for 5 minutes to elute DNA	00:05:00
15	Confirm DNA presence by Nanodrop and place in 4°C refrigerator	00:00:00

Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2–5 min after addition of Buffer PE

Linked Resources

- DNA Gel Extraction (Qiagen) (Protocol)
- DNA Gel Extraction (Qiagen) (Protocol)

Experiment: **2015-08-27 Ligation Check**

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Ura3 Recombination
| Owner: Jarrod Shilts

Signed by Jarrod Shilts on September 04, 2015 at 18:45

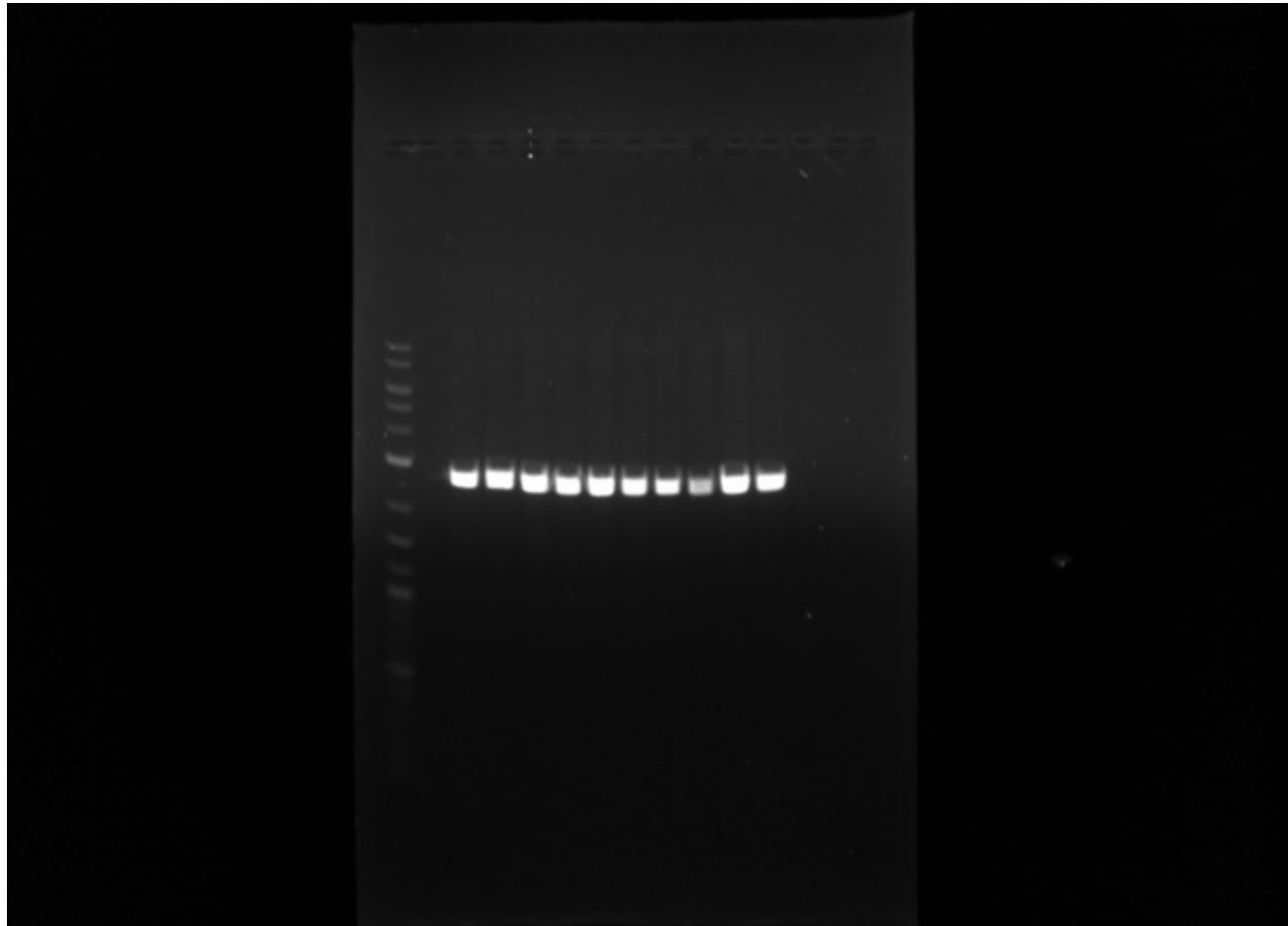
Experiment Procedures

New Procedure

Steps

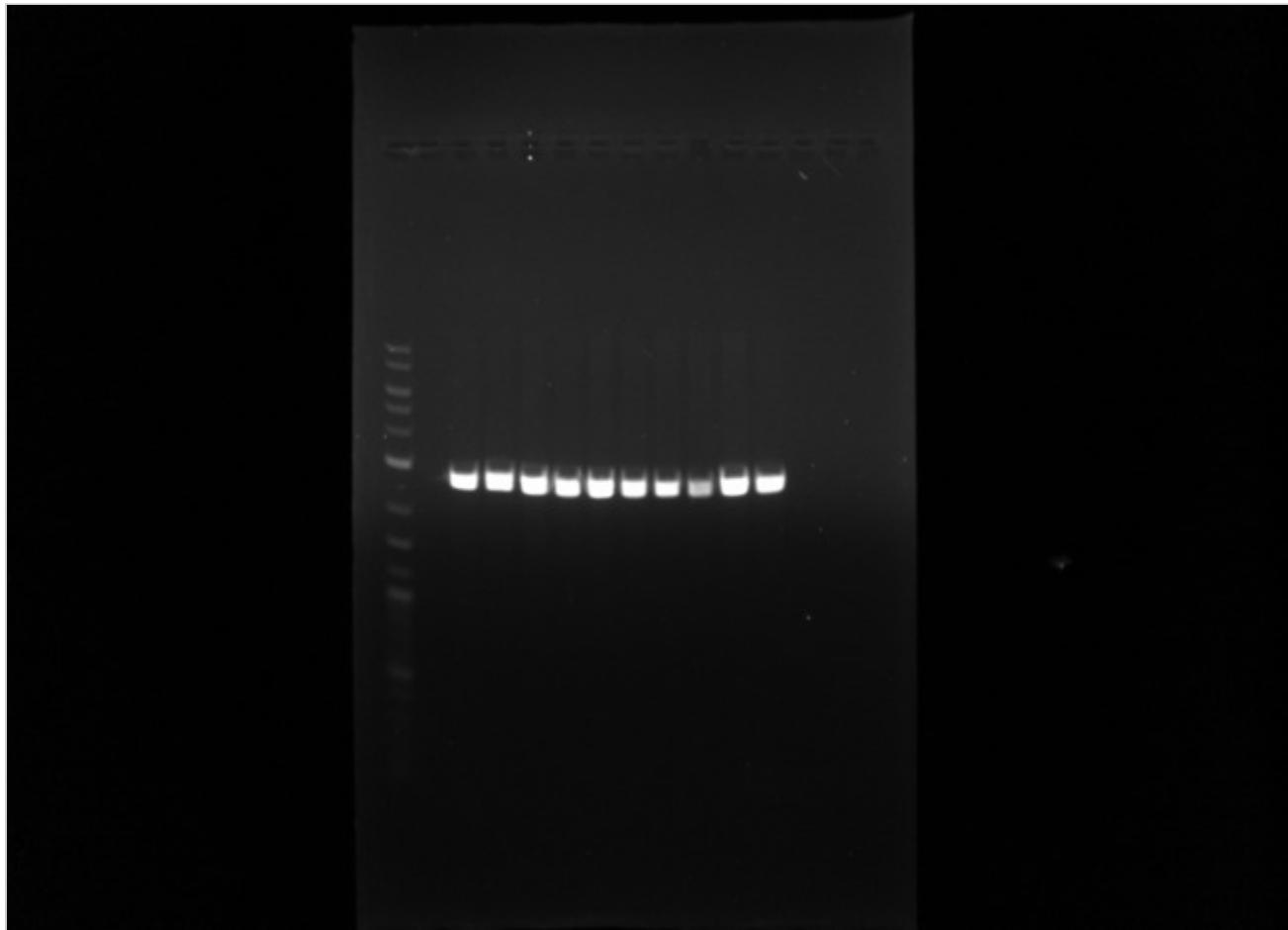
#	Title	Timer
1	Grow overnight cultures of ten colonies	00:00:00
2	Miniprep cultures	00:00:00
3	Digest EcoRI in 10ul	00:00:00
4	Run on gel	00:00:00

Experiment Results



No ligation of Ura3 into pUC19

Experiment Attached Images



15-08-27_ura3.tif

Experiment: 2015-09-03 Second round ura3 PCR

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: Ura3 Recombination
| Owner: Jarrod Shilts

Experiment Procedures

New Procedure

Steps

#	Title			Timer
1	50 ul Q5 polymerase reaction with 0.5 ul of gel-extracted PCR template from #3,4,5,6			00:00:00
2		Template	FWD primer	REV primer
	3a	#3	T7A_HR_puc19	T7A_HR_gal1_with_ura
	4a	#4	T7A_HR_with_ura	T7_HR_with_puc19
	5b	#5	T7A_HR_with_puc19	T7A_HR_gal1_with_ura
	6b	#6	T7B_with_ura3	T7_HR_with_puc19
3	Run all PCR on gel and extract band at ~0.5 bp (faint but present on all)			00:00:00

Experiment: **2015-09-01 first round ura PCR**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **Ura3 Recombination**
| Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

#	Title				Timer
1	50 ul platinum taq reaction with 1 ul genomic DNA alpha 199 or plasmid DNA				00:00:00
2		Template	FWD	REV	00:00:00
	#1	alpha 199 yeast DNA			
	#2	alpha 199 yeast DNA			
	#3	T7A (B)			
	#4	T7A (B)			
	#5	T7B (B)			
	#6	T7B (B)			
3	Gel extract bands with Qiagen kit. Elute 20 ul.				00:00:00

Milestone: **FPG Oxidation Assay**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Owner: **Jarrod Shilts**

Experiment: **2015-07-09 FPG methylene blue Test**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **FPG Oxidation Assay**
| Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 100 ng of San4 plasmid to PCR tube. Adjust volume to 5 ul with water. Add 1 ul of 100 mM methylene blue solution	00:00:00
2	Expose two tubes to white light from Macara lab dissecting microscope at setting #6 (maximum) for 30 minutes. Keep two tubes in dark, aside from a brief (<5 min) exposure to white light	00:00:00
3	To each tube, add 1 ul of 10x NEBuffer 1, 0.1 ul of 100x BSA. Add either 2.4 ul of water and 0.5 ul of FPG, or 2.9 ul of water. Incubate reaction at 37 degrees overnight	00:00:00
4	Add loading dye and run 10 ul on gel	00:00:00

Experiment Results

No DNA migration, likely caused by effect of positively charged methylene blue.

Experiment: **2015-07-14 FPG methylene blue**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **FPG Oxidation Assay**
| Owner: **Jarrod Shilts**

Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 2.3 ul (200 ng) of K546546 plasmid to 2.2 ul water. Add 0.5 ul of 100 mM methylene blue in dark box.	00:00:00
2	To light exposed samples, tape directly to bright fluorescent lamp on bench for 1 hour. Keep dark samples in box.	00:00:00
3		00:00:00

Experiment Results

Presence of methylene blue in sample interferes with DNA electrophoresis. Precipitate DNA first or try different oxidant

Experiment: 2015-07-16 FPG peroxide oxidation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: FPG Oxidation Assay
| Owner: Jarrod Shilts

Signed by Jarrod Shilts on July 18, 2015 at 22:21

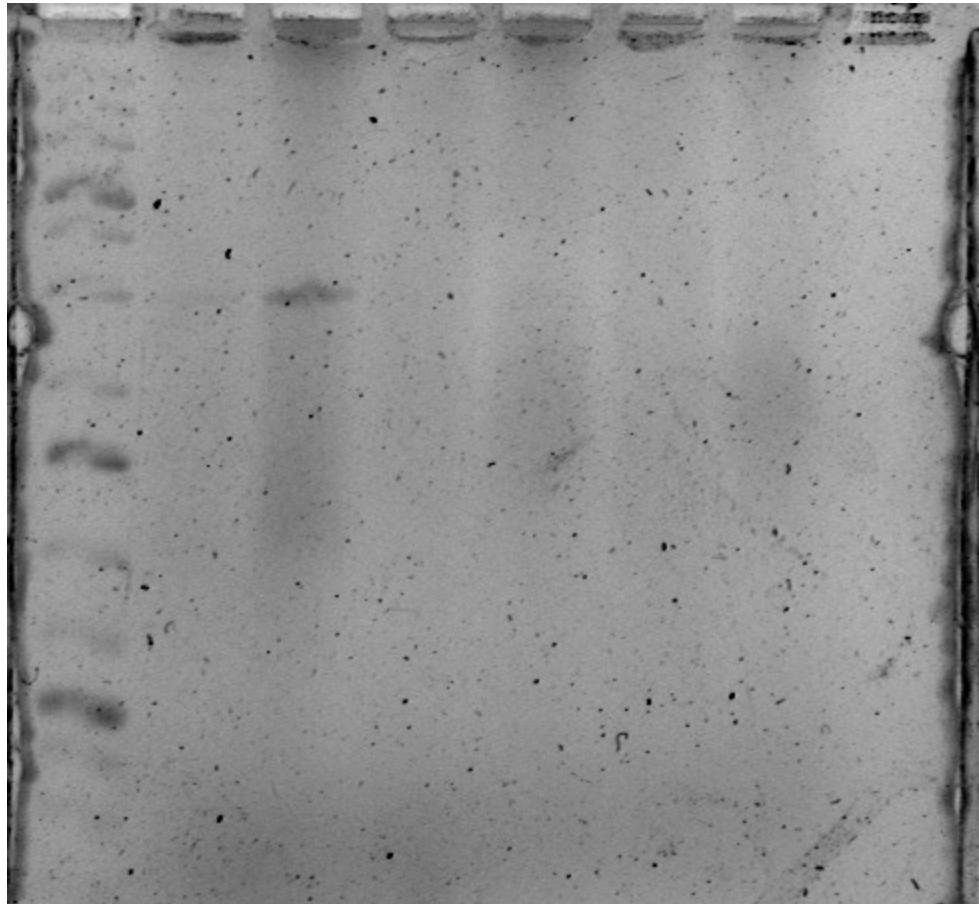
Experiment Procedures

New Procedure

Steps

#	Title	Timer																																				
1	To PCR tube, add 200 ng (2 ul) of E1010 plasmid DNA and the following. Low oxidation is final concentration of 100 uM	00:00:00																																				
	<table border="1"> <thead> <tr> <th></th><th>O-E- low</th><th>O+E- low</th><th>O-E+ low</th><th>O+E+ low</th><th>O-E- high</th><th>O+E- high</th><th>O-E+ high</th><th>O+E+ high</th></tr> </thead> <tbody> <tr> <td>Water</td><td>3 ul</td><td>1.5 ul</td><td>3 ul</td><td>1.5 ul</td><td>3 ul</td><td>1.5 ul</td><td>3 ul</td><td>1.5 ul</td></tr> <tr> <td>Oxidizing agents</td><td></td><td>1 ul FeSO4 (150 mM)</td><td></td><td>1 ul FeSO4 (150 mM)</td><td></td><td>1 ul FeSO4 (150 mM)</td><td></td><td>1 ul FeSO4 (150 mM)</td></tr> <tr> <td></td><td></td><td>0.5 ul H2O2 (1 mM)</td><td></td><td>0.5 ul H2O2 (1 mM)</td><td></td><td>0.5 ul H2O2 (8.82 M)</td><td></td><td>0.5 ul H2O2 (8.82 M)</td></tr> </tbody> </table>		O-E- low	O+E- low	O-E+ low	O+E+ low	O-E- high	O+E- high	O-E+ high	O+E+ high	Water	3 ul	1.5 ul	Oxidizing agents		1 ul FeSO4 (150 mM)			0.5 ul H2O2 (1 mM)		0.5 ul H2O2 (1 mM)		0.5 ul H2O2 (8.82 M)		0.5 ul H2O2 (8.82 M)													
	O-E- low	O+E- low	O-E+ low	O+E+ low	O-E- high	O+E- high	O-E+ high	O+E+ high																														
Water	3 ul	1.5 ul	3 ul	1.5 ul	3 ul	1.5 ul	3 ul	1.5 ul																														
Oxidizing agents		1 ul FeSO4 (150 mM)		1 ul FeSO4 (150 mM)		1 ul FeSO4 (150 mM)		1 ul FeSO4 (150 mM)																														
		0.5 ul H2O2 (1 mM)		0.5 ul H2O2 (1 mM)		0.5 ul H2O2 (8.82 M)		0.5 ul H2O2 (8.82 M)																														
2	Incubate at room temperature overnight	00:00:00																																				
3	Add to each reaction 3.4 ul of water, 1 ul 10x NEBuffer 1, and 0.1 ul 100x BSA. Add either 0.5 ul of water or 0.5 ul of FPG enzyme. Incubate at 37 degrees for 16 hours	00:00:00																																				
4	Dissolve 0.55 g of agarose in 45 ml of water by boiling. When flask cools to 50 degrees, add 5ml of 10x Alkaline gel buffer. Cast in cold room, let solidify 30 min, then submerge in 1x alkaline gel buffer for 1 hour.	00:00:00																																				
5	Add 4 ul of 6x alkaline gel loading dye and load the entire sample onto alkaline gel. Also load 4 ul of O'Gene ruler 1kb plus	00:00:00																																				
6	Run at 65 volts for 130 minutes at 4 degrees.	00:00:00																																				
7	Neutralize and stain with SYBR safe	00:00:00																																				

Experiment Results



Experiment Attached Images



15-07-18_oxidation_fpg.tif

Experiment: 2015-07-21 Plasmid Oxidation FPG Nick

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: FPG Oxidation Assay
| Owner: Jarrod Shilts

Signed by Jarrod Shilts on July 20, 2015 at 22:27

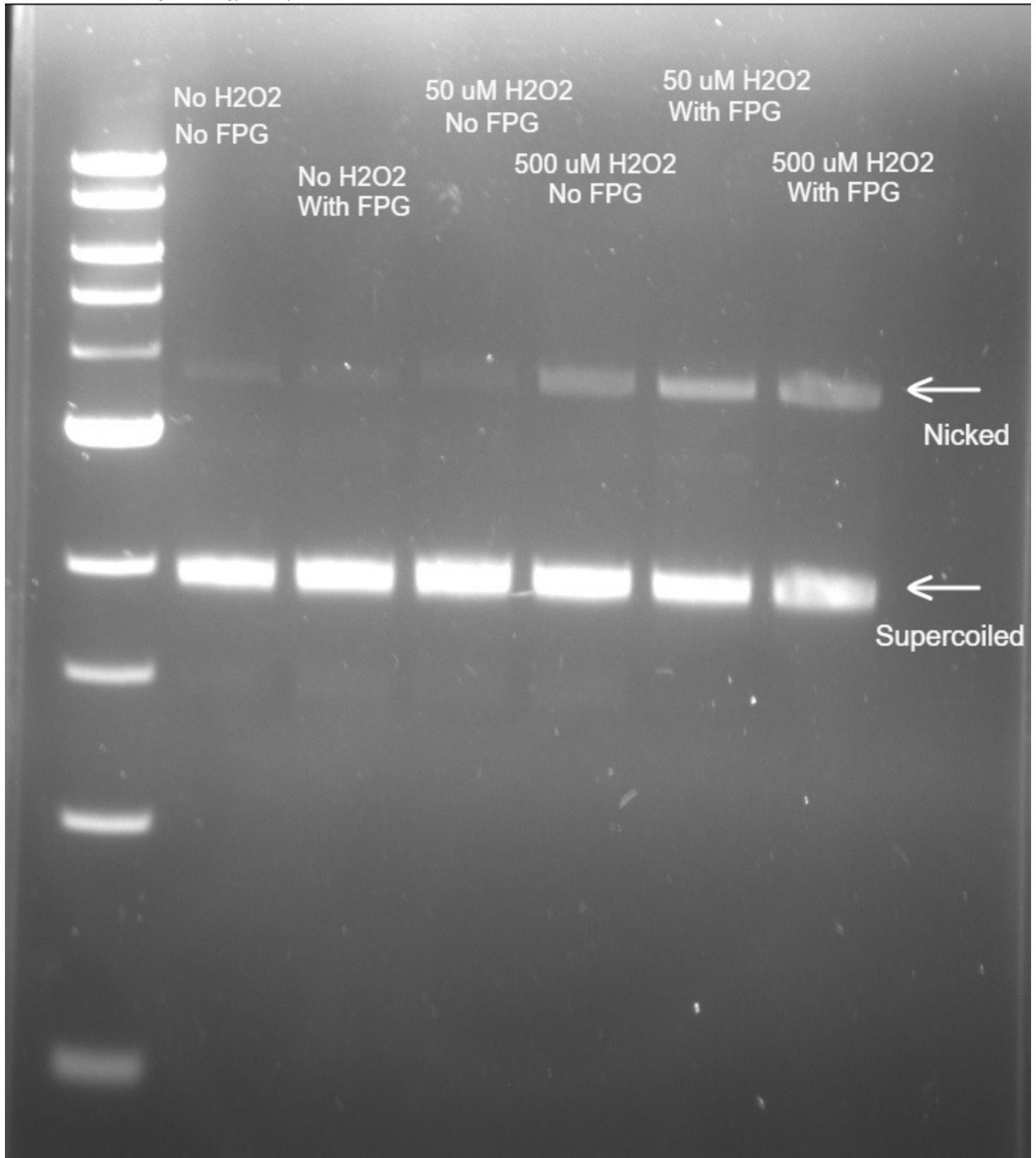
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Add 2.3 ul of R0082 (200 ng) with either 0, 1 (50 uM), or 10 ul (500 uM) of 1 mM hydrogen peroxide. Adjust volume to 16.8 ul with water. Incubate at room temperature 10 minutes	00:00:00
2	Add 2 ul of 10x NEBuffer 1, 0.2 ul of 100x BSA, and either 1 ul of FPG or water. Incubate 60 minutes at 37 degrees.	00:00:00
3	Immediately add 4ul loading dye and run 10 ul on 1.2% gel, 110 volts 45 min.	00:00:00

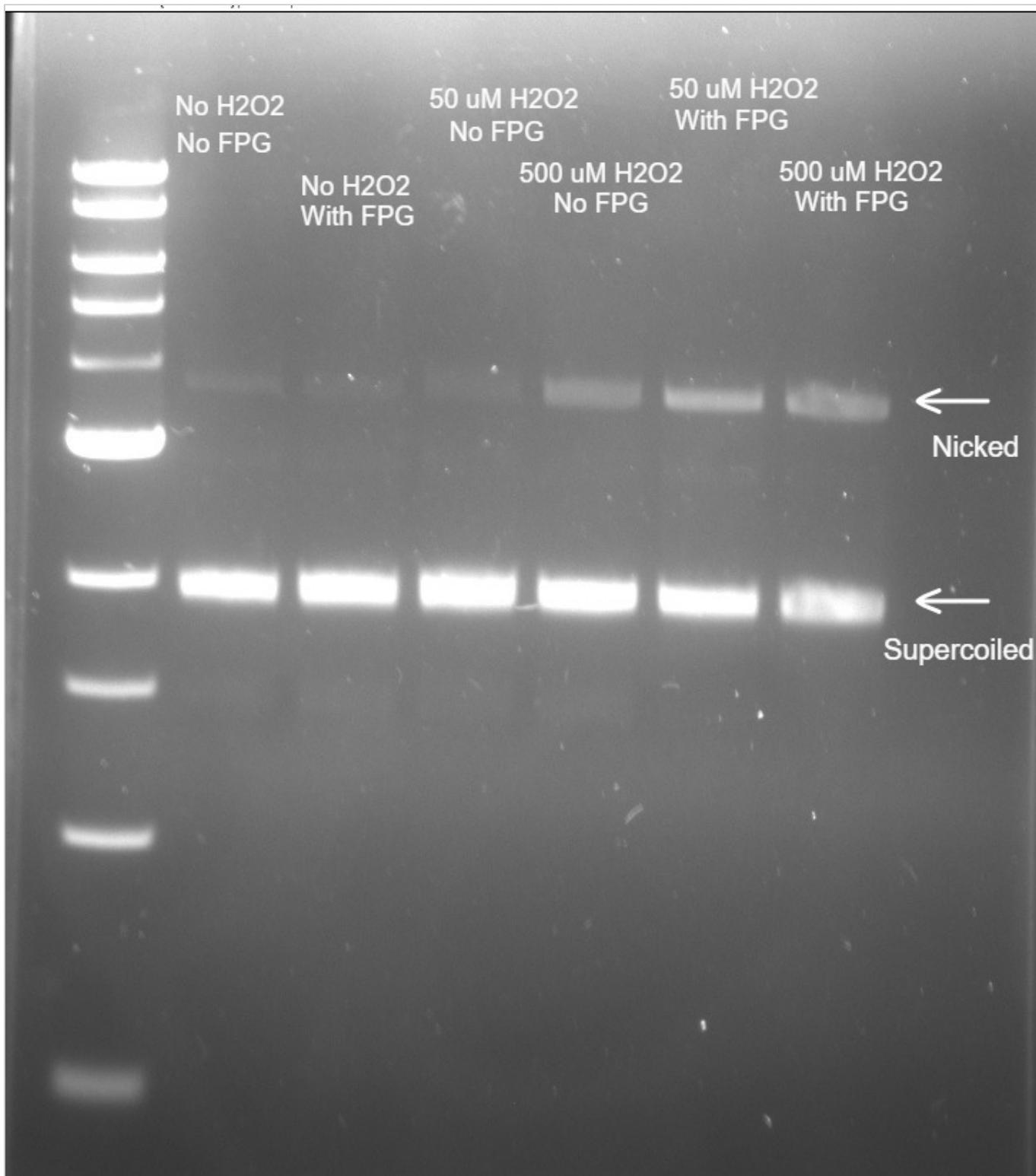
Experiment Results



Experiment Conclusion

Successful detection of oxidation with minimal background at dosage of 50 uM hydrogen peroxide

Experiment Attached Images



150720_plasmid_nick_fpg.jpg

Experiment: **2015-08-20 RFP ox oxidation assay**

Experiment Procedures

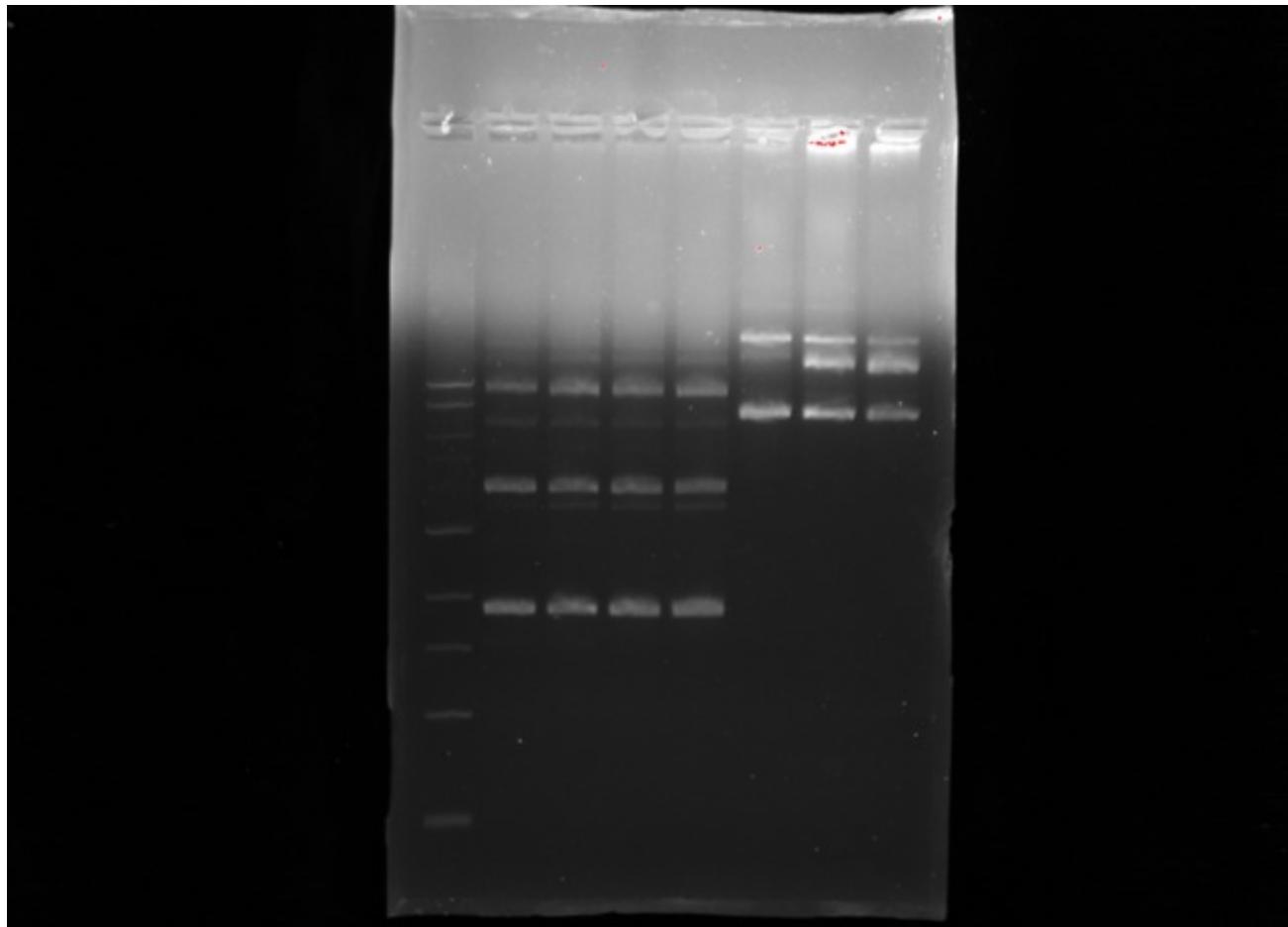
New Procedure

Steps

#	Title	Timer
1	Add 200 ng of RFP ox or E1010 RFP to a total volume of 15.8 ul. Add 1 ul of 1 mM H2O2 and incubate 10 min in dark (or add 1 ul water for neg control)	00:00:00
2	Add 2 ul NEBuffer 1, 0.2 ul 100 BSA, and 1 ul of FPG. Incubate 30 min at 37 degrees	00:00:00
3	Run 20 ul on 1% gel, 120 volts 60 min.	00:00:00

Experiment Results

Possible issue with plasmid used for E1010. Apparent molecular weights very far off, possibly from RecA BL21 strain used



Experiment Attached Images



150820_rfp_ox.tif

Experiment: **2015-08-22 fpg oxidation**

Project: **Modulating Evolutionary Potential with Rationally Designed Genes** | Milestone: **FPG Oxidation Assay**
| Owner: **Jarrod Shilts**

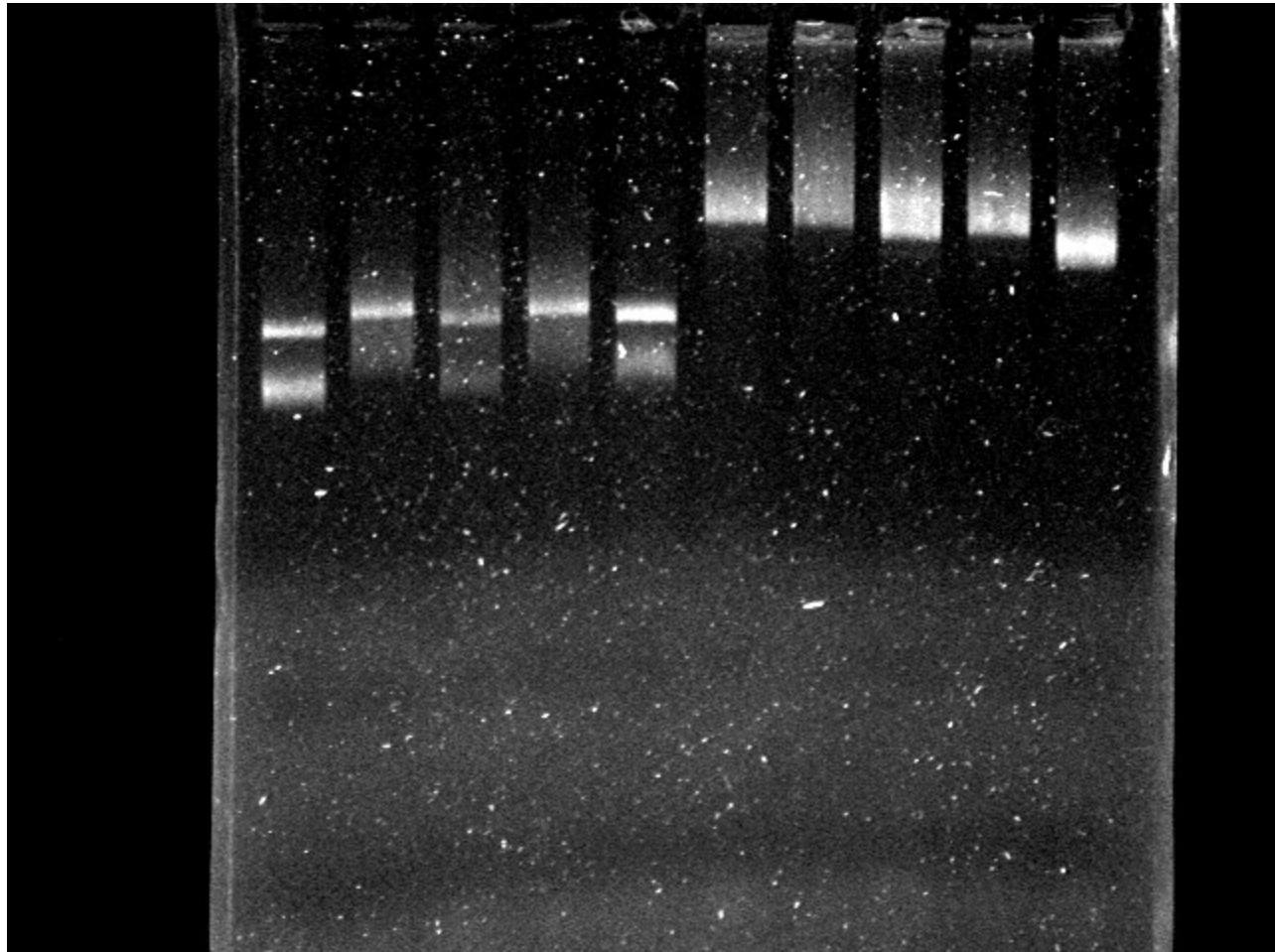
Experiment Procedures

New Procedure

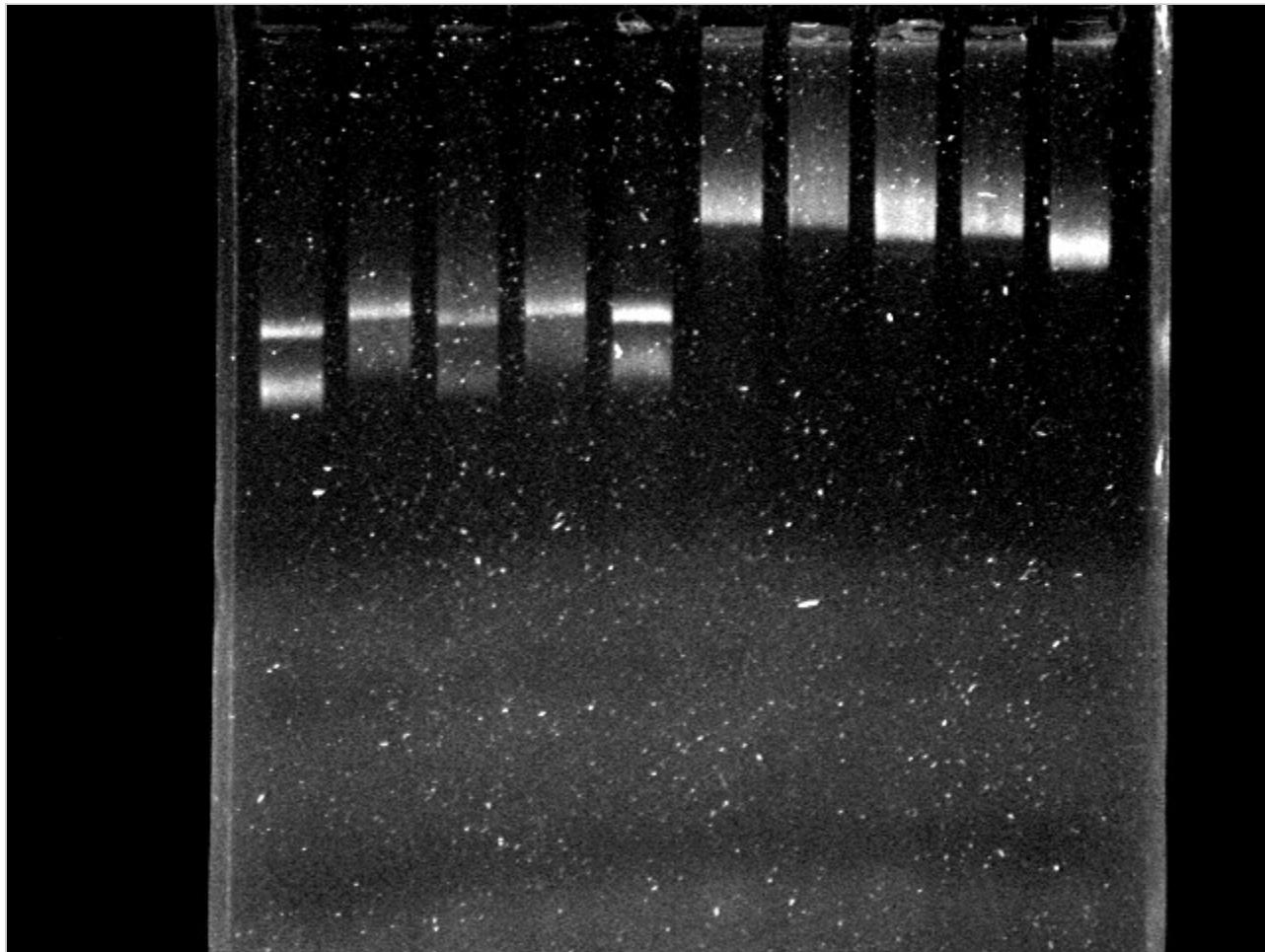
Steps

#	Title	Timer
1	Add 200 ng of plasmid DNA to total volume of 15 ul . Add 1 ul of 1mM H2O2 and incubate in dark at RT for 10 min	00:00:00
2	Add 2 ul NEBuffer 1, 0.2 ul 100x BSA, and 1 ul FPG. Incubate 37 degrees 30 min in dark	00:00:00
3	Run all of sample on 1% gel	00:00:00

Experiment Results



Experiment Attached Images



15-08-22_ox_fpg.tif

Experiment: 2015-09-04 RFPox oxidation

Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: FPG Oxidation Assay
| Owner: Jarrod Shilts

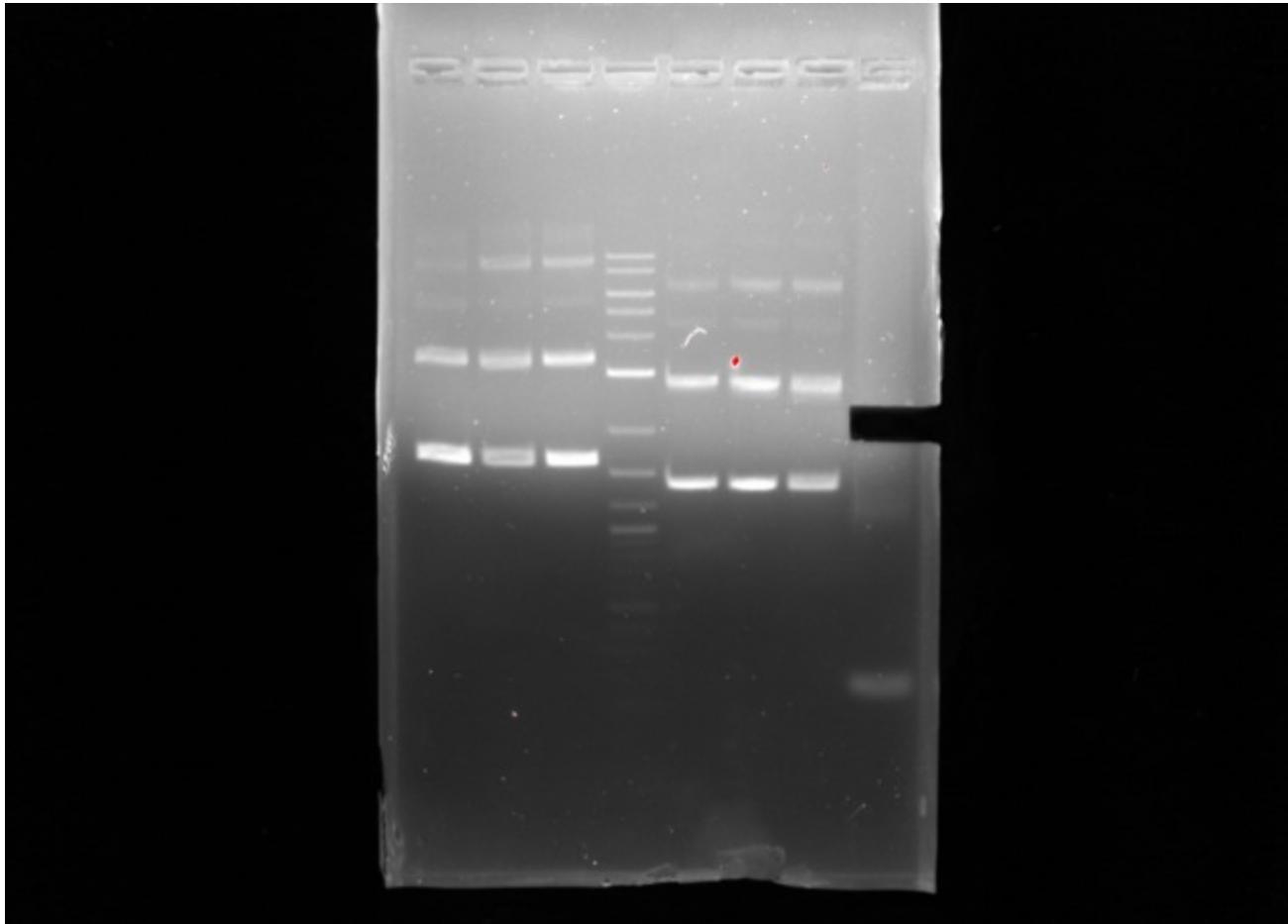
Experiment Procedures

New Procedure

Steps

#	Title	Timer
1	Dilute 8/22 E1010 and RFPOx pSB (A) to 100 ng/ul	00:00:00
2	Make mix of 44.5 ul water, 6 ul NEBuffer 1, 0.6 ul 100x BSA. Add 8.4 ul to six PCR tubes. Add 1 ul of DNA (100 ng). Add 0.5 ul of FPG (+) or water (-). Add 1 ul of 1 mM H2O2 to all	00:00:00
3	Incubate 37 degrees for exactly 30 min	00:00:00
4	Add 4 ul load dye and load onto 1% gel. Run 130 V 40 min	00:00:00

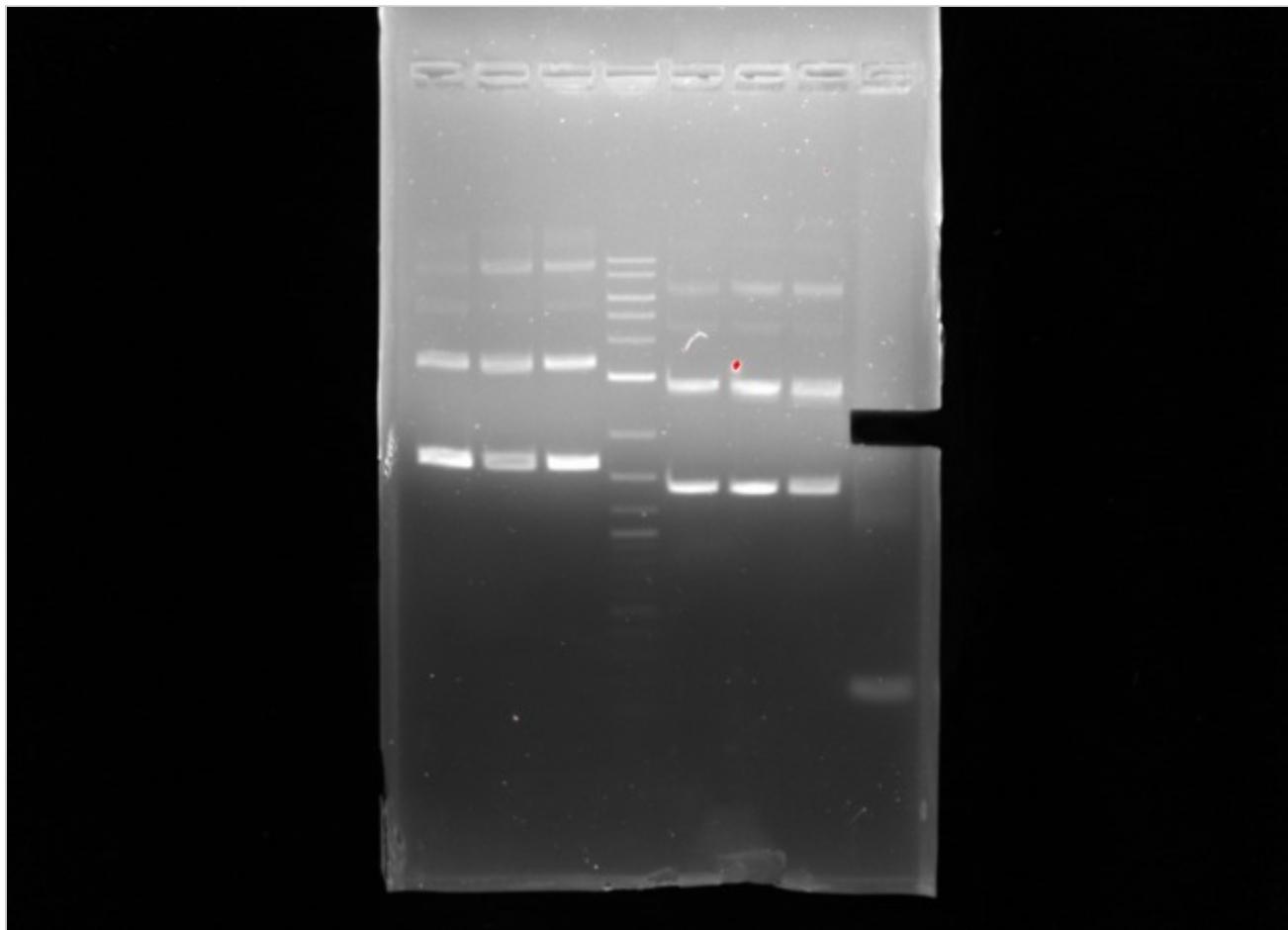
Experiment Results



identity of RFP ox (is no longer at correct size relative to E1010)

Issue with the

Experiment Attached Images



15-09-04_fpg_rfp_ox.tif

Experiment: 2015-09-05 Oxidation new RFP ox

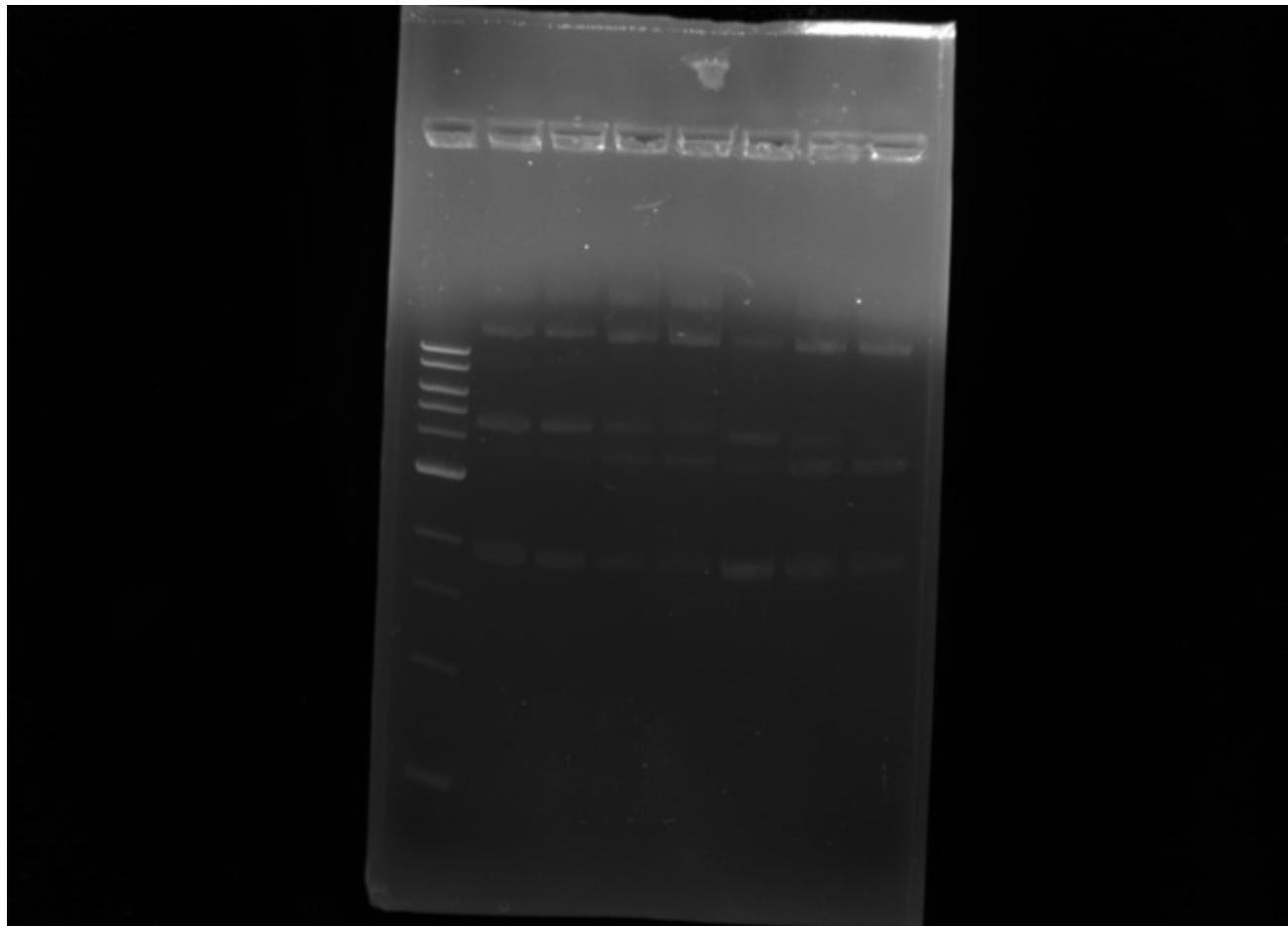
Project: Modulating Evolutionary Potential with Rationally Designed Genes | Milestone: FPG Oxidation Assay
| Owner: Jarrod Shilts

Experiment Procedures

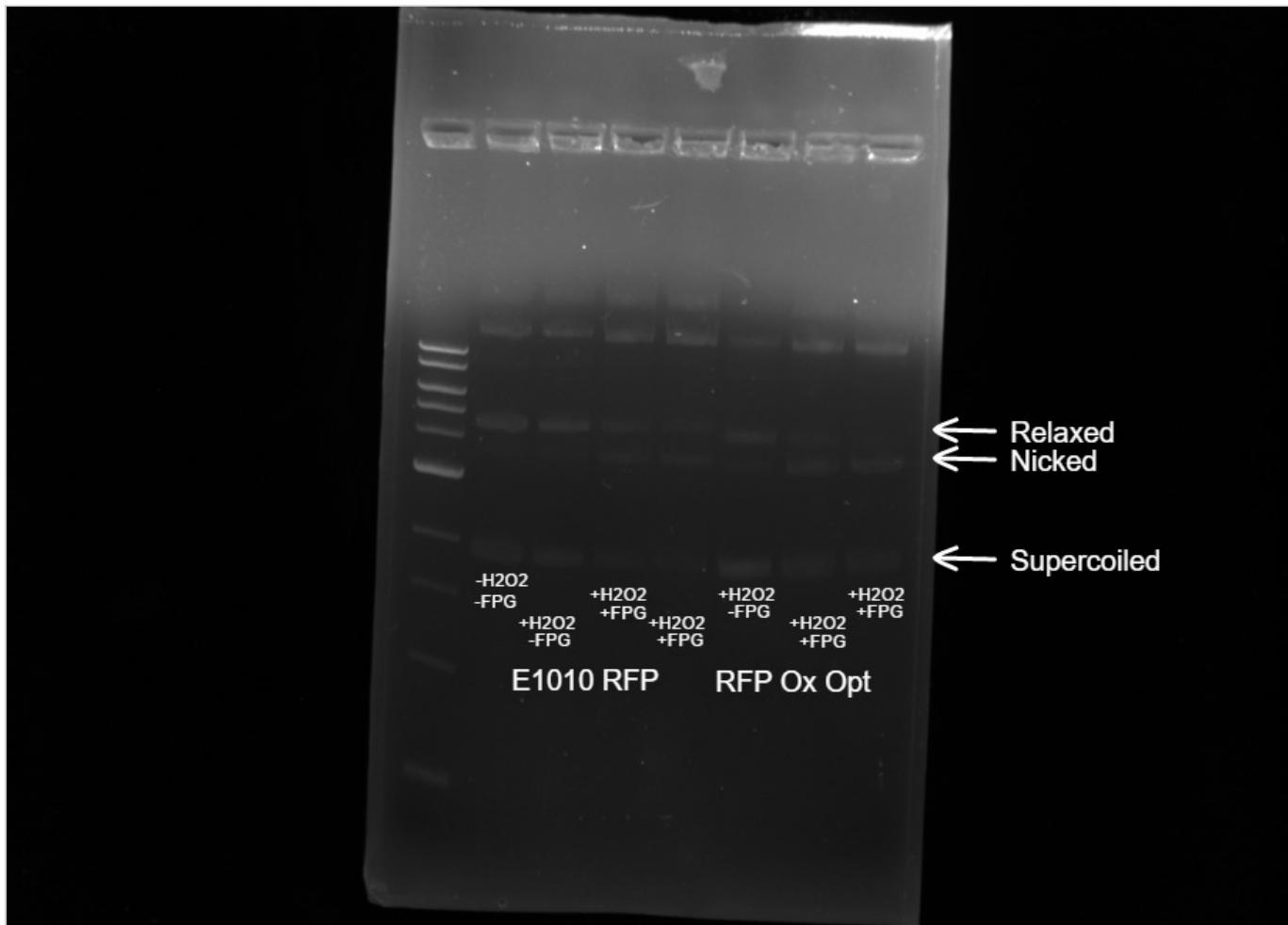
New Procedure

Experiment Results

new RFP ox extraction gives correct sizes. Increase in nicked form in control versus optimized



Experiment Attached Images



15-09-05_fpg_ox.tif